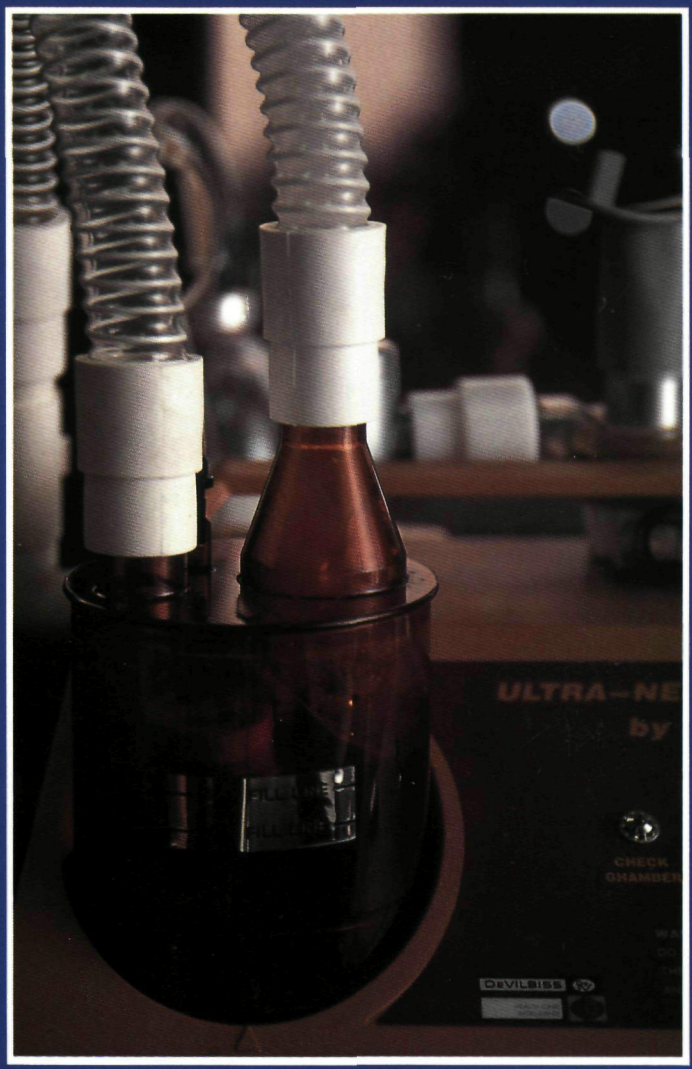
Bronchial Hyperresponsiveness to Ultrasonically Nebulized Distilled Water and Histamine in Asthmatic subjects



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CONTENTS

GENERAL INTRODUCTION AND AIM OF THE STUDY

1.1 BRONCHIAL HYPERRESPONSIVENESS: DEFINITION AND CLINICAL ASPECTS

Asthma is a disease characterized by paroxysms of dyspnea, wheezing and/or cough in a varying degree from indetectible mild to very severe [1]. In asthma an increased responsiveness of the tracheobronchial tree to a variety of stimuli is a characteristic feature. This phenomenon of bronchial hyperresponsiveness can be defined as an exaggerated bronchoconstrictor response of the airways on exposure to a small quantity of non-specific stimuli which do not not provoke such a reaction in normal subjects [2]. In contrast to a specific, allergic stimulus, the bronchoconstrictor response in non-specific bronchial hyperresponsiveness is not related to one particular stimulus, but to a variety of stimuli [3,4]. Bronchial hyperresponsiveness has become of main interest in the investigations of the underlying pathophysiological mechanisms and pharmacological treatment modalities in asthma [2,5]. Several studies have made clear that there seems to be a relation between the severity of bronchial hyperresponsiveness and the clinical prognosis of asthmatic subjects [6-11]. The presence of bronchial hyperresponsiveness in childhood is a risk factor for the development of chronic obstructive pulmonary disease (COPD) in adulthood [6,10,12]. Bronchial hyperresponsiveness in adult COPD patients is associated with an accelerated longitudinal decline in lung function [7-9,11]. Therefore, the assessment of bronchial hyperresponsiveness and farmacological modulation is very important in the treatment of asthmatic patients.

The non-specific stimuli that can cause bronchusobstruction in susceptible patients are presented in table 1. These stimuli are used in bronchoprovocation tests, to assess the relationship between a given dose of inhaled stimulus and the change in lung function caused by it. This relationship reflects the magnitude of bronchial hyperresponsiveness.

Bronchial hyperresponsiveness can lead to respiratory symptoms like dyspnoea and wheezing. Also coughing without bronchoconstriction can occur as the sole effect of bronchial hyperresponsiveness [13]. Variations in bronchoconstriction during day and night are a well-known phenomenon, especially late at night and early in the morning an increase in bronchoconstriction can be found [14]. Ryan et al. [14] demonstrated that in

asthmatic patients there was a close relationship between diurnal variation in peak expiratory flow rate and the PC_{20}histamine, the concentration of inhaled histamine causing a 20% fall in FEV_1. This threshold is used to measure the degree of bronchial hyperresponsiveness. In this study reversibility of bronchoconstriction caused by bronchodilator drugs also correlated with the histamine threshold [14]. Other investigators, however, could not reproduce these findings [15,16].

Table 1: Pharmacological, physical and chemical agents causing bronchusobstruction in asthmatic subjects.

PHARMACOLOGICAL STIMULI:	histamine [39], methacholine [39], acetylcholine [4], carbachol [40], propranolol [41], leukotrienes [42,43], prostaglandins [44], adenosine [45], platelet-activating factor [46], neurokinin A [47], bradykinin [48].
PHYSICAL STIMULI:	exercise [36], hyperventilation [32], cold air [33], fog [4], distilled water [34], hypertonic solutions [34].
CHEMICAL STIMULI:	SO_2 [49], ozone [50], citric acid [4].

Sleep-related symptoms are often seen in asthma and at the same time an increase in bronchial hyperresponsiveness has been established [17,18]. Martin et al. demonstrated an increased cellular inflammatory response in the bronchoalveolar lavage fluid during the night in patients with nocturnal asthma [19]. Also decreased plasma levels of adrenaline during the night and increased vagal tone have been suggested as cause of nocturnal bronchoconstriction [20].

Subjective parameters derived from a questionnaire of pulmonary symptoms, however, showed a poor relationship with the objective parameters such as lung function and bronchial hyperresponsiveness to histamine and methacholine [21-23]. In the diagnosis and treatment of bronchial asthma, therefore, assessments of bronchial hyperresponsiveness and lung function measurements are crucial.

1.2 ASSESSMENT OF BRONCHIAL HYPERRESPONSIVENESS

Bronchial hyperresponsiveness is usually assessed with histamine or methacholine inhalation challenges [24]. Both histamine and methacholine provocation tests have been well standardized [25-27] and therefore have been shown to be reproducible and sensitive tests in the diagnosis and assessment of disease severity in asthmatic subjects [27-29]. Since bronchial hyperresponsiveness is caused by a complex pathophysiologic process in the airway wall, a challenge test with histamine and methacholine presumably only detects certain components of the mechanisms underlying bronchial hyperresponsiveness. The histamine- and methacholine-induced bronchoconstrictor response is mainly the result of a direct effect of these agents on the airway smooth muscles [24]. Physical stimuli, however, may assess a more complete pathway of bronchial hyperresponsiveness. Exposure to exercise and distilled water results in the release of mediators in the airway wall leading to airway smooth muscle contraction [30]. Pauwels et al. suggested the distinction of direct and indirect stimuli in bronchial hyperresponsiveness measurements, reflecting the direct or indirect effect of these stimuli on the airway smooth muscles [24].

An advantage of the physical, indirect agents is the similarity to naturally occurring non-specific stimuli in daily life, and therefore a probably better correlation with day-to-day symptoms in asthma has been suggested [24,27]. Another advantage of these physical stimuli is that the bronchoconstriction they induce is specific for asthma. Patients without asthma do not react to these stimuli [31-34], whereas they do react to pharmacological ones. For if an appropriate dose of inhaled histamine or methacholine is administered, normals react with bronchoconstriction [35].

The main purpose of this thesis is to determine whether in clinical studies on bronchial hyperresponsiveness in asthma, a physical stimulus like ultrasonically nebulized distilled water (UNDW) will have advantages over histamine inhalations. In contrast to exercise, bronchoprovocation with inhaled distilled water offers the opportunity of constructing a dose-response curve and calculating a threshold, i.e. PD_{20}UNDW as described in detail in chapter 4 [34,36]. This makes a good comparison with the PD_{20}histamine, the histamine threshold dose which may be concidered as the golden standard for bronchial responsiveness, possible. Another advantage of UNDW challenge

is that the test is not limited by the locomotive system of the patient, in contrast to exercise testing. Furthermore, bronchoprovocation with UNDW only last a relatively short time, is well tollerated by the patients, and easy to perform [37,38]. Therefore, we have choosen bronchoprovocation with UNDW as an indirect stimulus to investigate bronchial hyperresponsiveness in comparison with histamine.

1.3 AIM OF THE STUDIES

In this thesis several aspects of bronchial hyperresponsiveness to ultrasonically nebulized distilled water and histamine in asthmatic subjects, with respect to clinical investigations, are described.
The aims of the investigations presented in this thesis are:

1. To determine whether in clinical studies on bronchial hyperresponsiveness in asthma, a physical stimulus like ultrasonically nebulized distilled water will have advantages over a pharmacological stimulus like histamine.

2. Validation of the histamine provocation test using a new dosimeter (Jaeger APS), to investigate the reproducibility of this test and to compare this technique with the 2-minute tidal breathing technique. (chapter 2)

3. To validate the method of UNDW provocation for the assessment of bronchial hyperresponsiveness in asthmatic subjects. To investigate several aspects of this method: the reproducibility of the test, the shape of the dose-response curves, the duration of the distilled water-induced bronchoconstrictor effect and its relationship with the histamine-induced bronchoconstriction in asthmatic subjects (chapter 4).

4. To investigate the interaction of subsequent histamine and UNDW provocation tests and whether inhalation of histamine before UNDW-induced and histamine-induced

bronchoconstriction can cause refractoriness for one or both of these stimuli (chapter 5).

5. To compare the protective effect of the inhaled muscarine receptor antagonist ipratropium bromide on the UNDW-induced bronchoconstrictor response with the β_2-agonist terbutaline. (chapter 6).

6. To compare the effects of long-term treatment with the anti-inflammatory drugs beclomethasone and nedocromil sodium on bronchial hyperresponsiveness to UNDW and histamine, and the effects on lung function and asthma symptom scores in allergic asthmatic subjects. Moreover, it is investigated whether the UNDW and histamine challenge tests are comparable to assess changes in bronchial hyperresponsiveness. (chapters 7 and 8).

7. To investigate the relationship between asthma symptom scores and bronchial hyperresponsiveness to histamine and UNDW, and to determine whether the differences in the underlying mechanisms of the bronchoconstrictor responses to histamine and distilled water are reflected in these relationships. (chapter 8)

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HISTAMINE PROVOCATION TEST WITH THE APS DOSIMETER: REPRODUCIBILITY AND COMPARISON TO THE TWO-MINUTE TIDAL BREATHING TECHNIQUE.

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Submitted for publication

2.1 ABSTRACT

The Asthma Provocation System (APS) by Jaeger (Würzburg, FRG) is a breath-actuated dosimeter frequently used in routine lung function laboratories for the assessment of bronchial hyperresponsiveness. We investigated the reproducibility of the histamine provocation test with the APS system in nine asthmatic subjects. The repeated histamine provocation tests on two different days showed a good reproducibility with a standard deviation for repeated measurements of 10.4%. The APS dosimeter technique was further compared with the 2-minute tidal breathing technique with the Wright nebulizer. In fifteen patients suspected for bronchial hyperresponsivenesse a histamine challenge with the two techniques was performed on two different days. Both techniques showed a good correlation ($r=0.93$, $p<0.0005$) and equally distinguised hyperresponsive from non-hyperresponsive subjects. The limit of agreement (-103 % and +83 %) and 95% confidence limit ($\pm$27%) of the two techniques, however, show that the results of the APS dosimeter and tidal breathing technique do not completely agree with each other.

2.2 INTRODUCTION

In 1985, Jaeger (Würzburg, FRG) introduced the Asthma Provocation System (APS), a breath-actuated dosimeter designed for accurate dosage in bronchial challenges. During inspiration the aerosol is delivered instantenously after inspiration and a few maximal inspiratory capacity breaths are sufficient to deliver 45 μl of the aerosol. The APS is frequently used in Europe, and according to the information of the manufacturer, 60 to 70 per cent of all lung function laboratories in the Netherlands use the APS. However, the system has not validated yet. Therefore the aim of this study was to investigate the reproducibility of the histamine provocation test with the APS dosimeter. Another aim was to compare the histamine provocation test using the APS by the method described by Hargreave et al. [1], using a Wright nebulizer during 2-minute tidal breathing, a worldwide accepted and validated method of bronchial challenge.

2.3 PATIENTS AND METHODS

Subjects. The reproducibility of the dosimeter technique was investigated in nine patients with bronchial asthma [2], whose characteristics are shown in table 2.1.
On two different days the subjects performed a histamine provocation test with the APS dosimeter.

The comparison between the dosimeter and tidal breathing technique was investigated in fifteen patients suspected for bronchial hyperresponsiveness, whose characteristics are shown in table 2.2.

Table 2.1: Patient characteristics of the dosimeter reproducibility study in histamine provocation.

patient	sex	age (y)	FEV_1 (% pred)	$PD_{20}H_1$ (*) (μmol)	$PD_{20}H_2$ (μmol)	medication (**)
1.	M	50	60.5	0.02	0.01	s, ic
2.	M	41	57.7	0.10	0.05	s, ic
3.	M	35	80.7	0.14	0.25	s, ic
4.	F	21	100.6	0.25	0.39	s, ic
5.	F	26	75.2	0.31	0.50	s
6.	F	24	84.5	0.33	0.32	s
7.	M	25	91.9	0.62	0.32	s
8.	F	39	95.9	1.48	1.62	s
9.	F	30	101.6	1.78	1.59	s
mean		32.2	83.6	0.56	0.56	
SE		3.4	5.7	0.21	0.20	

(*) $PD_{20}H_1$ and $PD_{20}H_2$: The PD_{20} values of the histamine challenges on day 1 and day 2.
(**) s = salbutamol; ic = inhaled corticosteroids.

Table 2.2: Patient characteristics of the comparison of the dosimeter technique versus the 2-minute tidal breathing technique in histamine bronchial challenge.

patient	sex	age (y)	FEV_1 (% pred)	PD_{20} (*) (mg/ml)	PC_{20} (mg/ml)	medication (**)
1.	F	39	95.5	5.6	3.5	-
2.	F	53	84.6	12.6	9.1	-
3.	F	39	92.7	11.6	16.0	b
4.	F	61	109.4	0.02	0.02	ic
5.	M	15	77.0	0.22	0.32	b
6.	M	26	92.2	10.4	16.0	-
7.	F	73	54.8	2.2	2.5	b
8.	M	26	93.7	5.2	1.4	b
9.	M	27	76.7	0.80	0.36	-
10.	M	44	87.4	0.50	2.9	ic
11.	F	48	62.5	16.0	15.0	op
12.	F	47	101.3	8.4	13.2	-
13.	F	27	96.7	>16	>16	-
14.	M	47	67.9	>16	>16	-
15.	M	24	99.0	>16	>16	-
mean		39.1	86.1			
SE		4.0	4.0			

(*) 1 mg/ml histamine = 0.15 μmol histamine.
(**) b = β_2-agonist; ic = inhaled corticosteroids: op = oral prednisone;

On day 1 the subjects performed a provocation test with the dosimeter and on day 2 a provocation test with the 2-minute tidal breathing procedure. The baseline FEV_1 values on the two study days in both groups of patients were within 10% variation, all medication was stopped for a period of more than twelve hours before the test and the tests were performed at the same time of the day. None of the patients used theophylline. The protocol was approved by the Local Ethics Committee.

The APS dosimeter technique. The APS (Jaeger, Würzburg, FRG) (figure 2.1) consists of a breathing-unit, with a deadspace of 35 ml, in- and expiration valves, the Sandoz 1500 nebulizer with a very low variation in output and an expiration filter to collect the histamine aerosol. The output of the nebulizer is not affected by the liquid level in the nebulizer container. The aerosol particle size varies from 1.9 to 5.6 microns. The

mobile control unit adjusts the number of inhalations, the nebulization time, and the dosage interval. The compression unit consists of a high-pressure pump, an adjustable low-pressure reservoir for a constant nebulization pressure and a manometer. The pressure used in our investigation was 200 kPa. Depending on the output of the nebulizer container, six to eight maximal inspirations were used to deliver 45 μl of histamine per dose. The nebulization time was 0.6 seconds per breath and the aerosol was delivered within 0.1 second after the beginning of an inhalation. Inhalation of 45 μl of a concentration of 1 mg/ml histamine resulted in a dose of 0.15 μmol.

The tidal breathing technique. The aerosol was generated by a Wright nebulizer according to the method described by Hargreave et al. [1], with a nebulizer output of 130 $\pm$ 4 μl/min, at an airflow of 7 l/min. Subjects wearing a noseclip and face mask, inhaled the histamine for two minutes during tidal breathing.

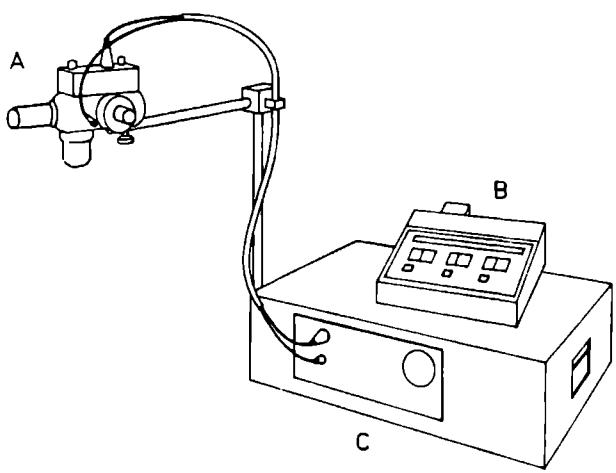

Figure 2.1: The asthma provocation sysytem consist of a breathing unit (A), a mobile controle unit (B), and a compression unit (C).

Provocation protocol. After inhaling of phosphate acid buffered saline, doubling concentrations of histamine acid phosphate (0.03 - 16.0 mg/ml) were administered at five-minute intervals. The test was stopped after the last dose of histamine, i.e. 16 mg/ml, had been inhaled or a fall in FEV_1 of more than 20% had been achieved. The PD_{20}histamine, the dose of histamine causing a 20% fall in FEV_1 from baseline values, was calculated for the APS method, whereas for the tidal breathing-method the PC_{20}histamine, concentration of histamine causing a 20% fall in FEV_1 from baseline values, was calculated. The PD_{20} and PC_{20} values were obtained from a semi-logarithmic dose-response curve by linear interpolation.

Lung function. Flow-volume curves were recorded on a pneumoscreen II (Jaeger, Würzburg, FRG), 30 and 90 seconds after inhalation. If a decrease of FEV_1 of 20% or more occurred the challenge was discontinued.

Statistical analysis. All data are presented as mean $\pm$ standard error (SE). The baseline FEV_1 is expressed as percentage of predicted [3]. The Student-t test was performed on the FEV_1 values. The PD_{20} and PC_{20} values were log transformed before further analysis. Correlations were calculated by the Pearson coefficient. The standard deviation for repeated measurements, limit of agreement and confidence intervals were calculated according to Bland et al [4]. Significance was accepted for $p<0.05$.

2.4 RESULTS

The baseline FEV_1 values on the two study days were not significantly different in either of the investigations.

Repeated histamine provocation tests performed with the APS dosimeter resulted in a mean PD_{20}histamine on day one of 0.56 $\pm$ 0.21 μmol and on day two of 0.56 $\pm$ 0.20 μmol (table 2.1). All PD_{20} values were within one doubling dose as shown in figure 2.2. The correlation between the repeated histamine provocation tests was $r=0.95$ and $p<0.0005$, with a standard deviation for repeated measurement of 10.4%.

In the study comparing the APS dosimeter with the 2-minute tidal breathing technique patients 1-12 showed a 20% fall in FEV_1 after inhaling of the last dose of histam-

ine, i.e. 16 mg/ml. The mean PD_{20}histamine and PC_{20}histamine in these patients were 6.1±1.6 and 6.7±1.9 mg/ml respectively. Those who responded to histamine (pat. 1-12) were used for calculating the correlation and limit of agreement between the two tests. Patients 13-15 did not respond. Both the dosimeter and tidal breathing technique could not induce a 20% fall in FEV_1 in these patients.

Figure 2.3 shows the correlation between the PC_{20} and PD_{20} values (r=0.93, p=0-.001). The limits of agreement for the PD_{20} compared to the PC_{20} are -102 % and +83%, with a 95% confidence interval of ± 27 %.

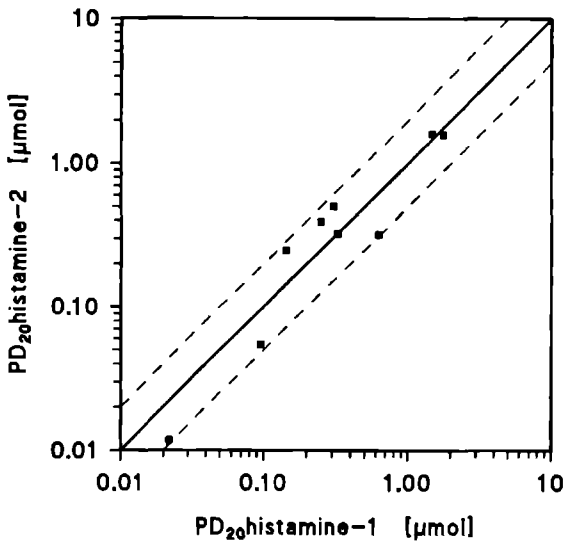

Figure 2.2: The reproducibility of the PD_{20}histamine performed with the APS dosimeter. The dashed lines represents the line of identity ± one doubling dose. All values are within one doubling dose. The standard deviation for repeated measurements is 10.4%.

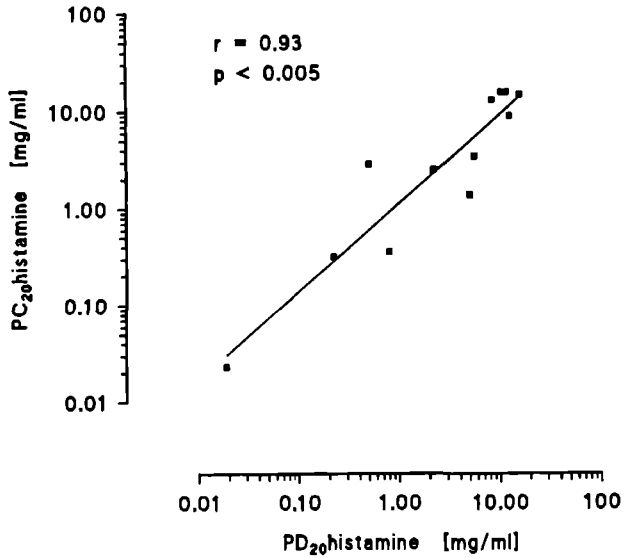

Figure 2.3: The correlation between the PD_{20}histamine, performed with the APS dosimeter and maximal inspiratory breaths, and the PC_{20}histamine, performed with the Wright nebulizer during two-minute tidal breathing.

2.5 DISCUSSION

The APS dosimeter technique showed a good reproducibility. A high correlation and a low standard deviation for repeated measurements was found, with all PD_{20} values within one doubling dose when the test was repeated in asthmatic subjects. The reproducibility of the APS technique is comparable to that of the 2-minute tidal breathing technique [5].

The data in this chapter show that both tests equally distinguished between responders and non-responders, subjects who did not reach a 20% fall in FEV_1 after inhaling 16 mg/ml histamine, and between hyperresponsive and non-hyperresponsive subjects, with the cut-off point of 8 mg/ml[1]. Furthermore, a good correlation exists between both test methods. However, it would be inaccurate to state that the APS, using a few maximal

inspirations, and the Wright nebulizer, using tidal breathing for two minutes, are fully comparable [4]. The limits of agreement between the two tests and the 95% confidence intervals show that both tests are not completely interchangeable. Ryan et al. [6] compared the French-Rosenthal dosimeter, using maximal inspiratory inhalation, with the Wright nebulizer, using tidal breathing for two-minute, and demonstrated a difference in deposition of the aerosol in the lung. Also differences in the shape of the dose-response curve have been found comparing the Wright nebulizer and the dosimeter technique, although the PC_{20} values of the two techniques correlated significantly [7].

The tidal breathing technique is a simple and inexpensive method, although the following factors should still be standardized to obtain reliable results: breathing frequency, flow rate, inspiration volume and breath holding time. These factors influence the inhaled dose in this method [8] while they are not constant during tidal breathing [9]. The dosimeter technique is a more sophisticated, more expensive method, which has the advantage that the inhaled dose of the agent is known exactly. The amount of drug delivered to the airways is also not influenced by the breathing pattern.

We conclude that the APS dosimeter technique is a good reproducible method and comparable to the tidal breathing technique with the Wright nebulizer, with regard to the reproducibility and assessment of bronchial hyperresponsiveness to histamine. The limits of agreement between the two techniques, however, show that the results are not completely interchangeable and must be interpreted carefully.

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ULTRASONICALLY NEBULIZED DISTILLED WATER-INDUCED BRONCHOCONSTRICTION IN ASTHMATIC SUBJECTS: REVIEW OF THE LITERATURE

3.1 INTRODUCTION

Bronchial provocation tests are standardized methods to measure and quantify bronchial hyperresponsiveness. Usually, inhalation tests with histamine or methacholine are used and their importance for the diagnosis and assessment of disease severity in asthma and chronic obstructive pulmonary disease has clearly been demonstrated [1-3].

Inhalation of non-isotonic aerosols can also cause bronchoconstriction in hyperresponsive patients as De Vries et al. demonstrated in 1964 in his test with cooled steam [4]. Steam was led into cold air to produce fog with a temperature of -10°C. Inhalation of this fog induced a bronchoconstrictor response in subjects with bronchial hyperresponsiveness [4].

After the introduction of the ultrasonic nebulizers in the 1960's, larger amounts of aerosols could be nebulized and deposited in the airways. In high-frequency ultrasonic nebulizers with a transducer frequency of above 0.5 mHz, the output is a function of the input power to the transducer, but little is known about factors which influence the droplet size in the aerosol [5].

In the first decade, ultrasonic nebulizers were used for inhalation of drugs by asthmatic patients and patients with cystic fibrosis [6], and for humidification of air in mechanical ventilators [7]. In 1980, however, Allegra et al. [8] introduced ultrasonically nebulized distilled water (UNDW) as a bronchial provocation test in asthmatic patients and since then several effects of hypotonic and hypertonic aerosols in normal subjects and in patients with asthma have been published [8-13].

In the following paragraphs the literature on UNDW bronchoprovocation tests is reviewed and the mechanisms underlying the bronchoconstriction induced by inhalation of UNDW are discussed. Different techniques of nebulization are described as well as the correlation with other bronchoprovocation tests and the factors related to the refractory period. Furthermore, the method of the UNDW challenge test used in the studies presented in this thesis is described, including the reproducibility, the duration of the bronchoconstrictor effect and the correlation to histamine challenge.

3.2 HYPOTHESIS WITH RESPECT TO THE MECHANISMS OF UNDW-INDUCED BRONCHOCONSTRICTION

The mechanism of bronchoconstriction induced by inhalation of UNDW has been extensively studied but has not been elucidated yet [11,14]. In contrast to isotonic solutions, inhalation of both hypotonic and hypertonic aerosols can induce bronchoconstrictor responses in asthmatic patients [11,14]. In normal subjects inhalation of aerosols of these solutions only induces some coughing but not bronchoconstriction [11,14,15]. The non-isotonicity of the inhaled aerosol appears to be a prerogative to elicit the bronchoconstrictor response [11]. Eschenbacher et al demonstrated that for hyperosmolar solutions an increase in ion concentrations is an additional stimulus for bronchoconstriction [11]. Two solutions with an osmolarity of 1,232 mmol, one containing 4 % sodium chloride and one containing 18.3% dextrose and 1% sodium chloride, were inhaled by the same asthmatic subjects. Inhalation of the solution with the higher ion content shifted the dose-response curve to the left, thus increasing the sensitivity of the subjects to the bronchoconstrictor stimulus.

An increased acidity of the nebulized solution can significantly enhance the bronchoconstrictor potency of hypoosmolar aerosols [16]. Furthermore, the titratable acidity of the inhaled aerosol is important [17]. Inhalation of buffered solutions of HCl and H_2SO_4 induced a significant bronchoconstriction in 8 asthmatic subjects, whereas inhaled unbuffered HCl caused a bronchoconstrition in only one patient and unbuffered H_2SO_4 had no effect. The pHs of all solutions were identical [17]. Sulphuric acid and hydroxymethane-sulphonic acid, both of them common constituents of acid fogs, had no specific broncho-constrictor effects in asthmatics [18,19]. Alkaline aerosols inhaled by mild asthmatic subjects did not induce bronchoconstriction [20].

An increase in temperature of the nebulized solution from 20 to 37 °C may diminish the bronchoconstrictor effect of distilled water aerosols, but not of aerosols of hypertonic solutions [21]. This finding, however, has not been confirmed by other investigators [9]. The degree of bronchoconstriction induced by these aerosols is also influenced by the inspiratory flow rate, since increasing the inspiratory flow rate to 1.2 l/s with constant

volume diminished the UNDW-induced bronchoconstrictor response when compared to the flow rate at tidal breathing [22]. The increased flow rate probably changes aerosol deposition in the airways.

Bronchoconstriction induced by the inhalation of non-isotonic aerosols appears to be caused to a considerable degree by the osmolar changes in the airway epithelium. Acidity of the solution and titratability of the acid increases the bronchoconstrictor response, while the temperature of hypotonic aerosols may also be important.

Sheppard et al. [10] demonstrated a clear decrease in UNDW-induced bronchoconstriction after pretreatment with 0.2 and 2.0 mg of inhaled atropine sulphate in asthmatic subjects. The two doses of atropine caused a similar reduction in baseline airway resistance, but the higher dose was significantly more effective in reducing the UNDW-induced bronchoconstriction. Studies with inhaled ipratropium bromide at doses of 40 μg and 80 μg [8,23] did not show any effect of pretreatment with this muscarine receptor antagonist on UNDW-induced bronchoconstriction. High doses of ipratropium bromide, up to 2000 μg, could inhibit the UNDW-induced bronchoconstrictor response [24]. To investigate dose-dependency receptor blockade on UNDW response we studied the protective effects of 160 μg and 320 μg ipratropium bromide in 9 asthmatic patients. The results are described in chapter 5. Our data and the data reported in literature suggest at least a partial involvement of the parasympathetic nervous system and/or the muscarinic receptors on the airway smooth muscles in the UNDW-induced bronchoconstrictor response.

Although 4% lidocaine partially inhibits the coughing usually caused by inhalation of distilled water [11], it did not inhibit bronchoconstriction [10]. This, however, does not exclude involvement of afferent vagal nerves, since it is difficult to block all sensory nerve endings in the airways of asthmatic patients with inhaled local anaesthetics because of the risk of anaesthesia-induced bronchoconstriction in these patients [25]. Moreover, it has recently been shown in normal subjects, that the bronchodilator properties of inhaled capsaicin, a strong stimulator of vagal afferent nerve fibres, could be blocked by inhaled lidocaine, whereas the capsaicin-induced brochoconstriction was not influenced [26]. Stimulation of vagal afferent nerve endings can also cause a local axon reflex in the airway wall [27]. This induces the release of neuropeptides from sensory nerve endings with

both bronchoconstrictor and bronchodilator properties. These local axon reflexes may well play a role in UNDW-induced bronchoconstriction as well. Inhaled morphine can inhibit UNDW-induced bronchoconstriction in asthmatic subjects and this effect is correlated to atropine inhibitory effects, suggesting that morphine inhibits a vagally mediated bronchoconstrictor response to UNDW [28]. Opiate receptors are located throughout the central and peripheral nervous system, but the location at which morphine inhibits the UNDW-induced bronchoconstriction is uncertain [28].

At present, it seems that the autonomic nervous system of the airways is involved in UNDW-induced responses, but it is unclear to what extent.

The release of mediators from inflammatory cells plays an important role in the acute and late-phase asthmatic response [29]. In UNDW-induced bronchoconstriction these mediators also appear to be involved [30,31]. In vitro, it has been demonstrated that rat mast cells release preformed mediators like histamine and neutrophil chemotactic activity when suspended in a hypotonic solution [32]. Inhalation of UNDW in asthmatic patients causes an increase in blood neutrophil chemotactic activity, whereas no such change could be demonstrated in normal subjects [31]. Serum histamine levels were also elevated in these patients, but there was no significant difference in the serum histamine response between normals and asthmatics. Local hypoosmolar challenge to airway segments of four asthmatic patients by bronchoalveolar lavage through a flexible bronchoscope resulted in the release of histamine into the airway fluid, while lavage with normal saline did not have such an effect [33]. Pretreatment with terfenadine, a specific H_1 receptor-antagonist, could diminish the UNDW-induced bronchoconstrictor response [34,35], indicating that H_1-receptor activation by histamine plays a role in UNDW-induced bronchoconstriction. Pretreatment with disodium cromoglycate [9,14] and nedocromil sodium [36] completely blocks the bronchoconstrictor response to UNDW exposure in asthmatics. These drugs are supposed to inhibit mediator release from inflammatory airway cells [37], which indicates involvement of these cells in UNDW-induced bronchoconstriction. On the other hand, both drugs have so-called "non-mast-cell effects", as they inhibit vagally induced bronchoconstriction by inhaled sulphur dioxide in man [38].

Leukotrienes D_4 and E_4, arachidonic acid metabolites synthesized in inflammatory cells during asthmatic reactions, may also be important in the UNDW response, since

specific antagonists of these leukotrienes can diminish bronchoconstriction induced by UNDW. [39].

In table 3.1 summarizes the protective effects of different drugs on the UNDW-induced bronchoconstrictor response.

Table 3.1: Protective effects of drugs on the UNDW-induced bronchoconstrictor response.

drug	dose	time between dose and test	degree of protection	reference
sodium cromoglycate	10 mg	30 min	+	40
	12 mg	30 min	+	41
	20 mg	10 min	++	9
	20 mg	30 min	+	42
	40 mg	20 min	++	8
nedocromil sodium	4 mg	30 min	++	36
	8 mg	30 min	++	41
atropine	0.2 mg	15 min	-	10
	2.0 mg	15 min	+	10
	0.04 mg/kg	20 min	+	43
ipratropium bromide	40 µg	30 min	-	23
	80 µg	60 min	-	8
	160 µg	30 min	-	chapter 5
	320 µg	30 min	+	chapter 5
	200 µg	90 min	+	24
	1000 µg	90 min	+	24
	2000 µg	90 min	+	24
salbutamol	200 µg	60 min	++	8
	200 µg	2,4,6 hrs	+	44
	200 µg	4 hrs	+	45
fenoterol	200 µg	30 min	++	23
	200 µg	2 hrs	++	44
	400 µg	20 min	++	25
	400 µg	2,4,6 hrs	++	44
clenbuterol	40 µg	4 hrs	++	45
terbutaline	500 µg	30 min	+	chapter 5
theophylline	10 mg/kg*	4,8,12 hrs	+	46
nifedipine	20 mg*	45 min	±	25
	10 mg**	20 min	+	44

Table 3.1. (continued)

drug	dose	time between dose and test	degree of protection	reference
verapamil	12.5 mg	15 min	±	9
tiaramide	100 µg	1,2,4 hrs	++	9,10
	200 µg	,,	++	9,10
	400 µg	,,	++	44
chlorpheniramide maleate	6 mg	2 hrs	-	
	12 mg	2 hrs	-	44
ranitidine	25 mg	20 min	-	44
furosemide	40 mg	direct	++	47
indomethacin	100 mg*	2 hrs (3 days)	-	48
lidocaine	4%	direct	-	10
LY 171883 (leukotriene antagonist)	400 mg* (7 days)	2 hrs	+	39
morphine	0.15 mg\kg	30 min	+	28
terfenadine	120 mg*	4 hrs	+	34
	180 mg*	2½ hrs	+	35
	240 mg*	4 hrs	+	34

All medication was administered by inhalation, unless indicated otherwise. (* : medication used orally, ** : medication used sublingually).

Degree of protective effects:

- : no protection
± : protection in some patients
+ : significant protection for all patients
++ : totally blocked broncho-constrictor response.

In conclusion, the mechanism of UNDW-induced bronchoconstriction is probably multifactorial, including osmolar changes in the airway epithelium, mediators released from inflammatory cells, and vagal reflex mechanisms.

3.3 METHODS OF UNDW CHALLENGE

A number of different methods is used for bronchial provocation tests with ultrasonically nebulized aerosols. DeVries et al. demonstrated in 1964 bronchoconstrictor responses to cooled steam in asthmatic patients [4]. In 1980, Allegra and Bianco [8] introduced a standardized provocation test with UNDW. They challenged the patients with a single dose of aerosol with a nebulizer output of 2 ml/min. The aerosol was inhaled for 5 minutes through a face mask at tidal breathing. A modification of this test with multiple, increasing doses of aerosol, created the opportunity to construct a dose-response curve [44]. To achieve increasing doses the nebulization time was doubled at a fixed output of 2 ml/min. Consecutive nebulizations of 30, 60, 120, 240 and 480 seconds were given [44,49]. Schoeffel et al. [14] measured the volume of inhaled aerosol and administered increasing doses of distilled water by doubling the inhaled volume at a fixed output of 2 ml/min., and 5, 10, 20, 40, and 80 liter were given successively. Sheppard et al. [10] achieved increasing doses by doubling the output of the nebulizer at a fixed inhalation time of 3 minutes (0.4, 0.7, 1.5, 3.1 and 5.4 ml/min).

Thus, four different techniques are presently used for bronchial provocation tests with inhaled aerosols of distilled water. A comparison of the efficacy of these different techniques has not been performed. Differences in ventilation rate between subjects and during challenging within the same subject [50] suggest that volume-dependent doses used in bronchial challenge with ultrasonically nebulized aerosols are a reliable technique to construct dose-response curves [9]. We have therefore chosen a volume-dependent method of UNDW challenge in the studies presented here.

3.4 CORRELATION BETWEEN UNDW AND OTHER BRONCHIAL CHALLENGES

Several studies have been performed to investigate the relation between the UNDW provocation tests and other bronchial challenge tests. In this paragraph we shall

give a review of the literature on this issue and discuss the results.

Methacholine. In a group of 20 atopic asthmatic children no correlation could be found between the fall in FEV_1 induced by inhaled methacholine and UNDW [51]. Neither in a group of 25 asthmatic subjects ,aged 9-19, no correlation was found between PO_{20}UNDW, the nebulizer output of UNDW causing a 20% fall in FEV_1, and the PC_{20}methacholine, the concentration of methacholine causing a 20% fall in FEV_1 [52]. In a group of 15 asthmatic adults, a weak though significant correlation ($r=0.54$, $p<0.05$) was established between the PD_{20}methacholine and the PD_{20}UNDW [42]. In another group of 38 asthmatic aldults an also significant correlation was demonstrated between the methacholine and UNDW challenge ($r=0.62$, $p<0.0001$), with a threshold expressed as area under the curve [53]. Anderson et al. could not find a correlation between the PD_{20}methacholine and PD_{20}UNDW in a group of 15 asthmatic subjects [54]. However, when they increased the number of patients to 20 a significant correlation between PD_{20}methacholine and PD_{20}UNDW ($r=0.60$, $p<0.01$) was found [55]. In children, who seem to be less susceptible to UNDW-induced bronchoconstriction [51,56,57], the relationship between bronchoconstriction induced by distilled water inhalation and methacholine is probably different from that in adults.

Histamine. Fewer studies have been performed to compare the histamine- and UNDW-induced bronchoconstrictor response. In 20 asthmatic children sensitive to histamine, only five patients showed an UNDW-induced bronchoconstriction, and apparently no correlation was found [57]. Anderson et al. did not find a correlation between the PD_{20}UNDW and PC_{20}histamine in 16 adult asthmatic patients [54]. There was an interval of more than 24 days between the two tests in some patients, which may have contributed to the lack of correlation. Rosati et al., however, found a significant correlation ($r_s=0.45$, $p<0.001$) between histamine and UNDW challenge in 87 asthmatic patients [58].

Exercise. Several authors have investigated the relationship between exercise- and UNDW-induced bronchoconstrictor responses. In 17 atopic asthmatic subjects a significant correlation was found between the maximal fall in FEV_1 after exercise and the PO_{20}UNDW ($r=-0.66$, $p<0.01$), whereas in this group of patients no correlation was found between UNDW and methacholine challenge testing [52]. Bascom et al. also found a highly significant correlation between exercise and UNDW in 15 asthmatic subjects

(r_s=0.81,p<0.001), with a weaker correlation between UNDW and methacholine, and between exercise and methacholine (r_s=0.54 and r_s=0.49, respectively) [42]. In 7 asthmatic subjects no difference was found between the fall in FEV_1 after inhalation of UNDW and exercise [59]. Because of these correlations between UNDW challenge and exercise, and because of the similarrity in bronchoconstrictor mechanism between both stimuli (mast-cell-derived mediators play also an important role in exercise-induced bronchoconstrcition [60]) a common pathway for exercise- and UNDW-induced bronchoconstriction has been suggested [42,52,60]. In children, however, there is no relation between exercise and UNDW challenge, which casts some doubt on this hypothesis [56,57].

Hyperventilation with cold air. As the result of the studies investigating the influence of air temperature during exercise-induced bronchoconstriction [61,62], hyperventilation with cold air has been proposed as bronchial provocation test in asthmatic subjects. Comparison of the cold air hyperventilation- and UNDW-induced bronchoconstrictor responses showed a good correlation between the fall in FEV_1 after both stimuli in nine asthmatic patients (r=0.89,p<0.01) [63]. In another study of 11 asthmatic subjects, however, only a weak correlation was found between the PD_{20}UNDW and the minute ventilation causing a 20% fall in FEV_1 (r_s=0.51,p<0.05) [64]. In children no correlation has been established between cold air hyperventilation and inhalation of UNDW [51].

Hypertonic saline. No correlation was found between the PD_{20}UNDW and the PD_{20} for 4.5% saline [65].

Conclusion. We conclude that in adult asthmatic patients a correlation can be found between the methacholine- or histamine-induced bronchoconstriction and the bronchoconstrictor response to UNDW. Also exercise and cold air hyperventilation-induced bronchoconstriction are correlated to the UNDW-induced bronchoconstrictor effect. The correlations between exercise and UNDW are higher than the correlations between methacholine and UNDW, probably indicating a similar pathway for exercise- and UNDW-induced bronchoconstriction. No correlation was found between UNDW and hypertonic saline challenge, although a similar mechanism of bronchoconstriction for hypertonic saline and cold air hyperventilation has been proposed [66]. This needs further evaluation.

3.5 THE REFRACTORY PERIOD

Certain asthmatic patients responding to UNDW demonstrate a significant attenuated response after re-exposure to UNDW within several hours after the first challenge [9,67,68]. This phenomenon, known as refractoriness, has been demonstrated in provocation tests with other physical stimuli like exercise [69] and hypertonic aerosols [70]. In some provocation tests with pharmacological stimuli, this phenomenon can also be demonstrated and is called tachyphylaxis [71-73].

The mechanism of refractoriness has not been elucidated yet. Pretreatment with the cyclooxygenase inhibitor indomethacin can totally abolish the refractory period after repeated inhalation of UNDW [48]. This indicates that prostaglandins with bronchodilator properties such as PGE_2 are probably involved in the phenomenon of refractoriness. Mattoli et al. [67] found a correlation between the recovery rates of the FEV_1 after UNDW and methacholine challenge and the presence of refractoriness. Patients with a refractory period for UNDW recovered more rapidly from UNDW- and methacholine-induced bronchoconstriction than non-refractory patients [67]. In these patients bronchodilator mechanisms counteracting the bronchoconstriction may well be more pronounced or less affected by airway disease in asthma.

Increase in bronchial hyperresponsiveness after preinhalation of UNDW has been the subject of interest of several investigators. UNDW-induced bronchoconstriction followed by a histamine or methacholine provocation test, when the FEV_1 had spontaneously returned to the baseline value, showed an increase in bronchial hyperresponsiveness to histamine and methacholine [74], whereas hypertonic saline-induced bronchoconstriction showed no effects on bronchial hyperresponsiveness [75]. Furthermore, other authors found that the UNDW-induced increase in bronchial hyperresponsiveness to methacholine was related to the absence of a refractory period for repeated inhalation of UNDW [67]. The increase in bronchial hyperresponsiveness to methacholine after prior UNDW challenge waned off within two hours in the majority of patients, except for two out of ten patients, who developed a late-asthmatic reaction after UNDW challenge [12]. This late response was less severe than the initial response and the decrease in FEV_1 lasted four to five hours.

Eight hours after UNDW challenge there was still an increase in bronchial hyperresponsiveness to methacholine in the patients with a late-asthmatic reaction [12]. Prevention of the UNDW-induced bronchoconstictor response by preinhalation of cromolyn sodium also inhibitted the increase in bronchial hyperresponsiveness to methacholine [76].

Comparison of the refractory period after exercise and inhalation of UNDW showed a similar pattern of diminished bronchoconstriction in 14 asthmatic subjects [68]. Cross-refractoriness could be demonstrated if an exercise test was followed by an UNDW challenge, indicating a final common pathway in the mechanism of refractoriness for exercise and UNDW inhalation [68].

Preinhalation of histamine before exercise testing [77] or UNDW challenge [13] can also induce a refractory period, which will be discussed in detail in chapter 4.

Thus, refractoriness for repeated provocation tests with UNDW can be blocked by pretreatment with indomethacin, indicating involvement of the cyclooxygenase pathway and release of bronchodilator prostaglandins. The refractoriness is related to a more rapid recovery from the UNDW- and methacholine- induced bronchoconstrictor response. Refractoriness after inhalation of UNDW is similar to refractoriness after exercise, indicating a final common pathway. The absence of a refractory period after repeated UNDW challenge is related to an increase of bronchial hyperresponsiveness to histamine and methacholine following UNDW-induced bronchoconstriction.

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THE UNDW BRONCHOPROVOCATION TEST

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in part submitted for publication (paragraph 4.3 and 4.4)

4.1 THE UNDW BRONCHOPROVOCATION PROTOCOL USED IN THE PRESENT STUDIES

The bronchoprovocation test with UNDW in the present studies was performed according to a modification of the method of Schoeffel et al. [1]. To increase the sensitivity of the test the number of doubling doses were expanded. The interval between two consecutive doses was extended from 2 to 5 minutes as a result of a study of the duration of the UNDW-induced bronchoconstrictor response described in paragraph 4.4. The distilled water aerosol was generated by a Ultraneb 99 ultrasonic nebulizer (DeVilbiss, Somerset, USA). The level of distilled water in the nebulizer chamber was kept constant by means of a float and by continuous infusion of distilled water. The output of the nebulizer was fixed at 2.00 $\pm$ 0.05 ml/min aerosol generation in free space.

UNDW was inhaled at tidal breathing through a mouthpiece with tightened lips and nose clipped. A Leardal IV two-way valve (Stavanger, Norway), with a dead space of 24 ml, was placed between the aerosol hose and the mouthpiece. A respirometer (British Oxygen Company, London, UK) was connected to the expiratory port of a two-way valve to measure the total volume of inhaled air. After inhalation of 20 liters of ambient air through the system, doubling volumes of air with UNDW (3, 5, 10, 20, 40, 80, and 160 L) were inhaled at 5-minute intervals. Before and after the test the nebulizer chamber and aerosol hose were weighed and the total amount of inhaled distilled water was measured.

To assess bronchoconstriction, flow-volume curves were recorded 30, 90 and 180 seconds after inhalation by means of flow-volume equipment containing a pneumotachograph (Pneumoscreen II, Jaeger, Würzburg, FRG). If the FEV_1 at 180 seconds was lower than the FEV_1 values recorded at 30 or 90 seconds, further flow-volume curves were recorded until the FEV_1 increased spontaneously, in order to obtain the deepest fall in FEV_1. The test was stopped when a 20% fall in FEV_1 had been achieved or the last dose of air with UNDW, i.e. 160 l had been inhaled.

The PD_{20}UNDW, the cumulative dose of UNDW causing a 20% fall in FEV_1, was calculated from post-air values by linear interpolation on a semi-logarithmic dose-

response curve and was expressed in ml H_2O.

4.2 THE ULTRANEB 99

The ultrasonic nebulizer used in our studies was the Ultraneb 99 (DeVilbiss, Somerset, USA) shown in figure 4.1.

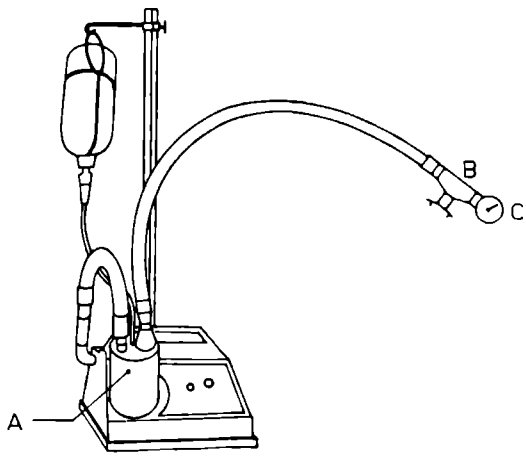

Figure 4.1: The ultrasonic nebulizer (A). The aerosol hose was connected to the inspiratory port of a two-way valve (B). A respirometer (C) was connected to the expiratory port to measure the volume of inhaled air with UNDW.

Specifications: transducer frequency 1.63 MHz
output: 0 - 7.5 ml/min
droplet size: 0.5 - 5.0 μ
median mass diameter: 2.8 μ
fan flow: 12 l/min

4.3 REPRODUCIBILITY OF THE UNDW-INDUCED BRONCHOCONSTRICTOR RESPONSE

Introduction

The dose-response curve describes the relationship between the dose of a stimulus and the stimulus-induced response. In bronchial challenge, the dose-response curve is often used to determine the degree of bronchial hyperresponsiveness in asthmatic patients [2]. Woolcock et al. investigated changes in the shape of the dose-response curve on histamine inhalation in asthmatic and normal subjects and demonstrated that the dose-response curves of asthmatic patients are shifted to the left in comparison with non-asthmatics. Moreover, there is an increase in the slope of the curve and the maximal bronchoconstrictor response (figure 4.2) [3].

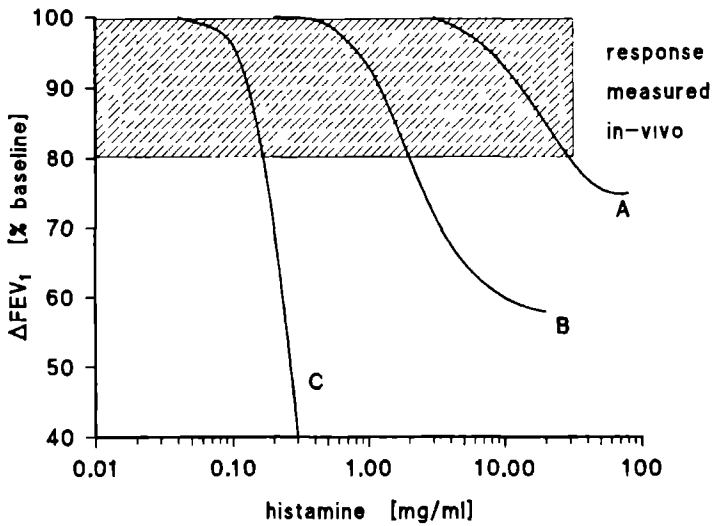

Figure 4.2: Dose-response curves of histamine-induced bronchoconstriction in normals (A), mild asthmatics (B) and more severe asthmatics (C), a modification to Woolcock et al. [3]. The shaded area indicates the part of the bronchoconstrictor response usually measured in vivo.

Theoretically, a distinction between pre- and post-junctional mechanisms has been proposed with regard to changes in the dose-response curve [2,4]. The leftward shift of the dose-response curve may well be the result of a pre-junctional mechanism, whereas an increase in maximal airway narrowing is suggested to be the result of a post-junctional phenomenon in the effector organ [2,4]. In clinical practice, the dose-response curves are usually limited to a 20% fall in FEV_1, indicated by the shaded area in figure 4.2, and no information is available with regard to the maximal obtainable level or plateau of airway narrowing. As mentioned above, Woolcock et al. [3] found that the more bronchial responsiveness increases, the steeper the slope of the dose-response curve and the higher the plateau of maximal airway narrowing on histamine are. This slope of the dose-response curve is theoretically identical to the reactivity, defined as the slope of the steepest part of the dose-response curve [5]. One may speculate that if there is a relation between the slope of the curve and the level of maximal airway narrowing, the reactivity of the curve may give some information about the maximal degree of airway narrowing.

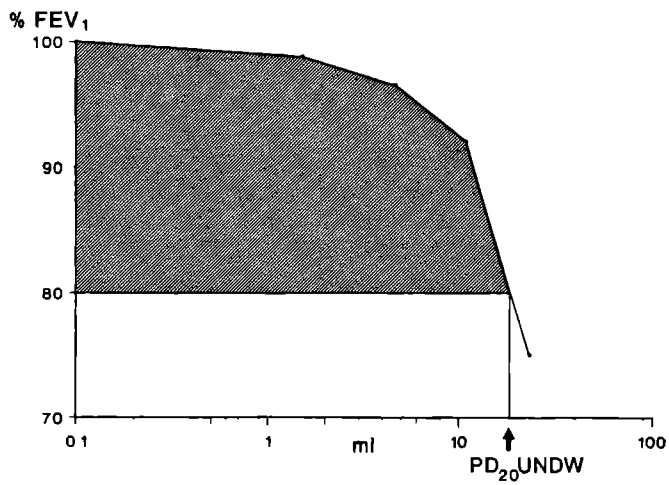

Figure 4.3: A dose-response curve of the UNDW-induced bronchoconstrictor response. The PD_{20}UNDW, the cumulative dose of inhaled distilled water causing a 20% fall in FEV_1, and the area under the UNDW dose-response curve, indicated by the shaded area are presented.

To quantify the UNDW-induced bronchoconstriction several thresholds such as PD_{20}UNDW [1], the area under the dose-response curve [6] and the maximal fall in FEV_1 [7] may be used. However, the shape of the dose-response is not expressed by these thresholds. We therefore measured both the sensitivity of the dose-response curve, which is a linear regression of the total dose-response curve [8], and the reactivity of the dose-response curve, which is the slope of the steepest part of the curve [5].

To validate the UNDW provocation test for the measurement of bronchial hyperresponsiveness in asthma we investigated the reproducibility of the UNDW-induced bronchoconstrictor response, with regard to the PD_{20}UNDW, the area under the curve, the sensitivity and the reactivity of the dose-response curve, and with regard to the relationships between these parameters.

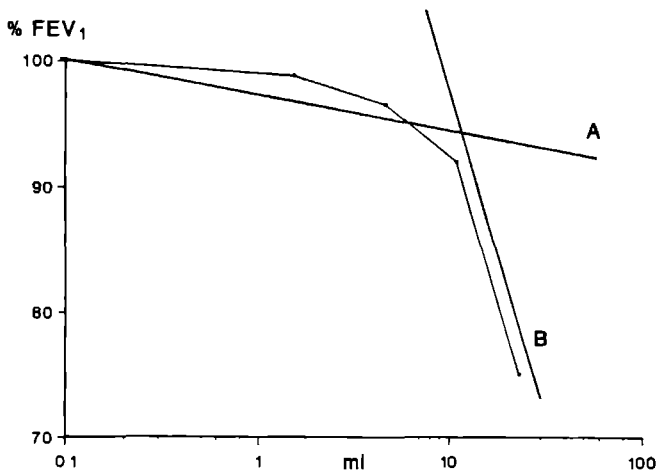

Figure 4.4: The sensitivity (A), the slope of a linear regression of the total dose-response curve, and the reactivity (B), the steepest slope of the dose-response curve are indicated on a UNDW-induced bronchoconstrictor response.

Patients and methods

Thirty-three stable asthmatics, with a reversibility of their baseline FEV_1 of more than 15 % after inhalation of a β_2-agonist and known responsiveness to UNDW inhalation, participated in the studies. Twenty-nine asthmatic subjects were atopic, defined as two or more positive skin reactions to a panel of common airborne allergens. The short-term reproducibility of the UNDW dose-response curve, within an interval of 3 weeks, was investigated in 17 patients, group A (Table 4.1).

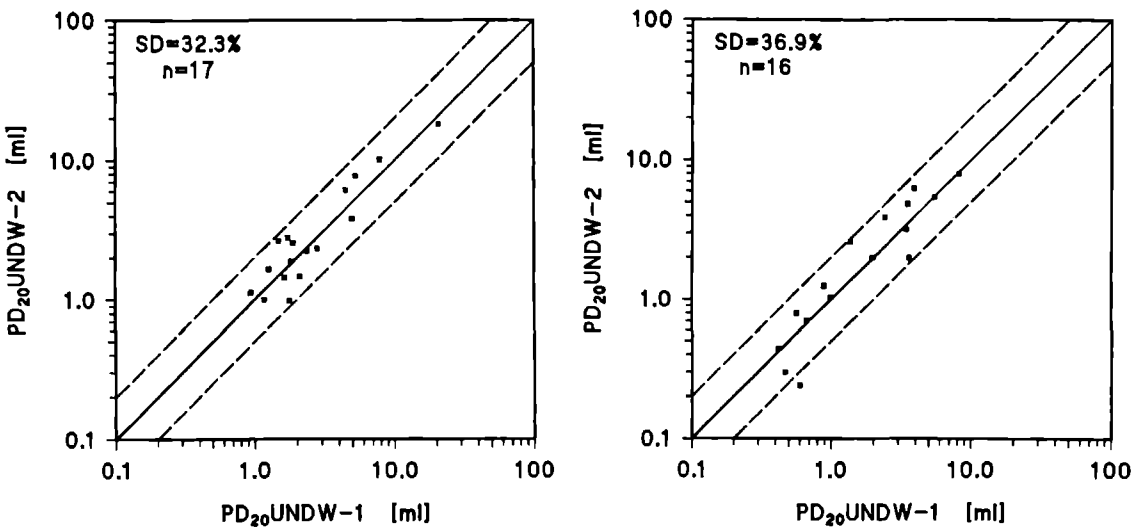

Figure 4.5: Short-term reproducibility (panel A) and long-term reproducibility (panel B) of the PD_{20}UNDW. The unbroken line is the line of identity, the broken line represents one doubling dose. The standard deviation for repeated measurements is 32.3% for short-term and 36.9% for long-term reproducibility.

Table 4.1: patient characteristics, group A

patient	age (y)	sex	atopic	FEV_1 (%pred)	PD_{20}UNDW (ml)	medication (*)
1.	54	F	+	71.0	1.3	s, c
2.	38	M	-	93.2	20.8	s, b
3.	34	M	+	90.0	1.5	s, b
4.	39	M	+	101.5	5.3	s, b
5.	45	M	-	96.0	2.4	s, b
6.	31	M	+	78.7	1.8	s, b
7.	46	M	+	54.2	0.9	s, b
8.	37	M	+	59.5	1.6	s, b
9.	18	F	+	83.5	2.1	s, b
10.	19	F	+	60.9	1.8	s, b
11.	39	F	-	100.0	1.9	s
12.	25	M	+	92.8	1.7	s
13.	30	F	+	101.6	4.5	s, bs
14.	26	F	+	69.1	7.8	s
15.	49	M	-	60.5	1.2	s, b
16.	21	F	+	97.2	5.0	s
17.	41	M	+	57.3	2.8	s, b
mean	34.8			80.4	2.3	
SE	2.6			4.2	0.5	

(*) s: salbutamol, b: beclomethasone, c: cromoglycate, bs: budesonide.

Twelve patients used inhaled corticosteroids, one patient cromolyn sodium and all subjects inhaled salbutamol regularly. The long-term reproducibility, with an interval of 2 to 3 months, was investigated in 16 atopic asthmatics, group B (Table 4.2).

Study design. All subjects performed an UNDW provocation test on 2 different days, at the same time of the day, with at least one day in between. The baseline FEV_1 on those days was within 10% variation. All medication was stopped for a period of more than eight hours before each test.

Table 4.2: patients characteristics, group B.

patient	age (y)	sex	atopic	FEV_1 (%pred)	PD_{20}hist (μmol)	PD_{20}UNDW (ml)
1.	25	M	+	41.8	0.01	0.6
2.	39	F	+	104.6	0.05	1.4
3.	23	F	+	86.6	0.002	0.6
4.	22	M	+	114.2	0.14	3.5
5.	27	M	+	88.5	0.16	2.0
6.	37	F	+	95.6	0.03	0.7
7.	31	M	+	73.9	0.17	3.4
8.	37	M	+	69.7	0.07	5.5
9.	50	F	+	92.6	0.04	2.4
10.	26	M	+	100.6	0.24	3.9
11.	31	F	+	77.5	0.002	0.4
12.	26	M	+	93.0	0.09	3.6
13.	20	F	+	97.9	0.004	0.5
14.	38	F	+	89.8	0.12	8.2
15.	16	M	+	64.9	0.04	1.0
16.	25	M	+	81.6	0.4	1.0
mean	29.5			85.9	0.10	2.4
SE	2.2			4.4	0.03	0.6

UNDW provocation test. The UNDW provocation test was performed as described in paragraph 4.1. A dose-response curve was constructed on a semi-logarithmic scale. To assess the threshold and shape of the dose-response we calculated the PD_{20}UNDW [9], the area under the dose-response curve (AUC) [6], the sensitivity [8] and the reactivity [5] (figures 4.3 and 4.4).

Lung function. Maximal expiratory flow-volume curves were recorded using the pneumoscreen II (Jaeger, Würzburg, FRG).

Statistical analysis. The reproducibility of the UNDW provocation test was calculated by a standard deviation (SD) for repeated measurements [10]. Multiple comparison was performed by the Wilcoxon signed rank test with Bonferroni correction. Correlations were calculated by the Spearman-rank test. All data were presented as means $\pm$ standard error (SE). Significance was accepted for $p < 0.05$.

Results

The baseline FEV_1 values on the days of measurement were not significantly different. The short-term reproducibility study was performed with a mean interval of 12.8 $\pm$ 2.4 days, the long-term reproducibility was performed with a mean interval of 94.9 $\pm$ 9.4 days. The mean values for PD_{20}UNDW, AUC, sensitivity and reactivity on the two study days, their correlation and SD for repeated measurements are presented in table 4.3. All parameters, except for reactivity, showed a good short-term and long-term reproducibility on the two study days. The PD_{20}UNDW values obtained during the short-term study were within one doubling dose, during the long-term study only ne exceeded this range (figure 4.5). Correlations between the different thresholds, i.e. PD_{20}UNDW, AUC, sensitivity and reactivity values are presented in table 4.4. The PD_{20}UNDW, AUC and sensitivity correlated well, whereas there was no correlation between these parameters and reactivity.

Discussion

The asthmatic subjects in this study were known to react to inhalation of UNDW. The PD_{20}UNDW, the sensitivity of the dose-response curve and the AUC correlated well, which indicates that these parameters represent a common characteristic of the dose-response curve. The PD_{20}UNDW and the sensitivity showed a somewhat lower SD value for repeated measurements than the AUC. The reactivity ,however, was not reproducible,

Table 4.3: Short-term and long-term reproducibility of parameters derived from the UNDW dose-response curve in asthmatic patients.

Short-term reproducibility (n=17)

	day 1	day 2	Spearman correlation	SD_{RM}
PD_{20}UNDW (ml)	3.8±1.2	4.0±1.1	r=0.78 p<0.005	32.3%
AUC	32.4±10.1	30.5±7.3	r=0.76 p<0.005	59.5%
S	-8.0±1.3	-8.1±1.3	r=0.82 p<0.005	43.9%
R	-31.3±3.1	-27.4±3.6	r=0.42 NS	54.0%

Long-term reproducibility (n=16)

	day 1	day 2	Spearman correlation	SD_{RM}
PD_{20}UNDW (ml)	2.4±0.6	2.7±0.6	r=0.92 p<0.001	36.9%
AUC	23.8±6.7	24.5±5.6	r=0.89 p<0.005	55.7%
S	-12.7±3.2	-12.2±3.1	r=0.89 p<0.005	31.6%
R	-80.9±52.9	-38.8±5.6	r=0.12 NS	-

PD_{20}UNDW: the cumulative dose of inhaled UNDW causing a 20% fall in FEV_1;
AUC: area under the dose-response curve;
S: sensitivity of the dose-response curve;
R: reactivity of the dose-response curve;
SD_{RM}: standard deviation for repeated measurements.

in contrast to the reactivity measured during histamine challenge [11] and it seems to be of no value, therefore, in assessing bronchial hyperresponsiveness to UNDW. The SD for

Table 4.4: Correlations between the PD_{20}UNDW, area under the curve, sensitivity and reactivity values of the UNDW dose-response curve in asthmatic subjects.

	AUC	S	R
Short-term interval			
PD_{20}UNDW (ml)	r=0.92 p<0.001	r=0.95 p<0.001	r=0.1 p>0.5
AUC	-	r=0.81 p<0.005	r=-0.2 p>0.4
S	-	-	r=-0.2 p>0.4
Long-term interval			
PD_{20}UNDW (ml)	r=0.97 p>0.001	r=0.99 p<0.001	r=-0.2 p>0.4
AUC	-	r=0.93 p<0.005	r=-0.3 p>0.25
S	-	-	r=-0.2 p>0.5

The presented values are the mean values of day 1 and day 2. PD_{20}UNDW: the cumulative dose of inhaled UNDW causing a 20% fall in FEV_1;
AUC: area under the dose-response curve;
S: sensitivity of the dose-response curve;
R: reactivity of the dose-response curve;

repeated measurements of the PD_{20}UNDW was 32.3% for short-term reproducibility and 36.9% for long-term reproducibility.

These results are comparable with reported and recalculated data on reproducibility for the UNDW provocation tests [9,12,13]. From data in literature we calculated SD values for repeated measurements of 23.8% [12] and 43.0% [13] for the short-term reproducibility. The SD for long-term reproducibility, determined in 12 patients, was 30% when the test was repeated within six months [9]. With regard to the PD_{20}UNDW, our data are comparable, therefore, with those reported in literature. The SD for repeated measurements of the PD_{20}histamine, as presented in chapter 2, revealed 10.4% for short-term reproducibility. As discussed in chapter 1, there is a clear difference in the mechanisms underlying bronchial hyperresponsiveness to histamine and UNDW. Histamine acts mainly directly on the airway smooth muscle, whereas UNDW inhalation a more complete pathway including mediator release in the airway wall [14,15]. This difference in underlying mechanism may explain the smaller SD for histamine challenge.

In conclusion, there is a good short-term and long-term reproducibility of the UNDW-induced bronchoconstrictor response in asthma, as measured with the PD_{20}UNDW, AUC and sensitivity. The PD_{20}UNDW and the sensitivity showed the least variation with regard to the SD for repeated measurements. In the further studies presented in this thesis we have therefore chosen the PD_{20}UNDW as threshold value to express changes in bronchial hyperresponsiveness to UNDW.

4.4 DURATION OF UNDW-INDUCED BRONCHOCONSTRICTOR EFFECT

Introduction

Few data are available on the time course of the UNDW-induced bronchoconstrictor response [7]. It is unknown whether these results can be applied to all methods of UNDW challenge. Therefore, we investigated the duration of the bronchoconstrictor

effect induced by inhaling increasing doses of air with UNDW, as described in paragraph 4.1.

Patients and methods

The duration of the bronchoconstriction after inhalation of the threshold dose of distilled water was investigated in 12 asthmatic subjects (Table 4.2, patient nos. 5-16). Their mean age was 30.8 $\pm$ 2.5 years with a mean baseline FEV_1 of 89.7 $\pm$ 3.6% of predicted; all patients were atopic.

UNDW provocation test. The UNDW provocation test was performed as described in paragraph 4.1.

Lung function. Maximal expiratory flow-volume curves were recorded with a pneumoscreen II (Jaeger, Würzburg, FRG). The duration of bronchoconstriction after reaching a 20% fall in FEV_1 was measured at 30, 90, 180 and 300 seconds after inhaling the last dose.

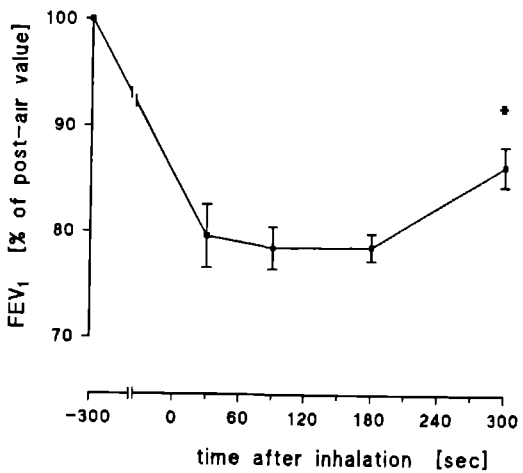

Figure 4.6: Duration of the bronchoconstrictor effect in 12 asthmatic patients after inhalation of the threshold dose of UNDW, causing a 20% fall in FEV_1, expressed as mean ($\pm$SE). There is a plateau of bronchoconstriction between 30 and 180 seconds after inhalation of the threshold dose.
** $p<0.01$ versus 90 and 180 seconds.*

Statistical analysis. Multiple comparison was performed by means of the Wilcoxon test with Bonferroni correction on all data. The FEV_1 was expressed as percentage calculated from baseline values. Data were presented as means $\pm$ standard error (SE). Significance was accepted for $p < 0.05$.

Results

The duration of bronchoconstriction after inhaling the UNDW threshold dose, i.e. the PD_{20}UNDW, is shown in figure 4.6.

The mean FEV_1 values 30, 90, 180 and 300 seconds after inhalation of the threshold dose were 79.6 $\pm$ 3.0, 78.5 $\pm$ 2.0, 78.6 $\pm$ 1.3 and 86.3 $\pm$ 1.9 % respectively, calculated of the post-air FEV_1 values. The mean FEV_1 values 30, 90, and 180 seconds after inhalation of PD_{20}UNDW-dose were not significantly different ($p>0.5$). At 300 seconds after UNDW inhalation the mean FEV_1 was significantly higher than the FEV_1 values at 90 and 180 seconds ($p=0.0076$ and $p=0.003$ respectively).

Discussion

The bronchoconstriction induced by inhalation of UNDW, as measured with the FEV_1, showed a plateau between 30 and 180 seconds after inhalation. Five minutes after inhalation the bronchoconstriction decreased. Our findings are in contrast with those of Allegra et al [7], who investigated the time course of UNDW-induced bronchoconstriction in 13 asthmatic subjects. They found a 300% increase in specific airway resistance above baseline 5, 10 and 15 minutes after inhalation of distilled water compared to saline inhalation, although they did not measure airway conductance between 0.5 and 5 minutes after inhalation. These differences in duration of UNDW-induced bronchoconstriction could be due to differences in method of provocation. Allegra et al. [7] challenged the patients with the inhalation of one dose of distilled water, i.e. 10 ml. In our protocol the

subjects inhaled doubling volumes of air with UNDW, starting with a dose of 3 liters of air with UNDW, which is equivalent to 0.2 - 0.5 ml H_2O, until a 20% fall in FEV_1 had been achieved. Thus, the inhaled dose of UNDW in our method is more adjusted to the specific, individual hyperresponsiveness, whereas in the method of Allegra et al. [7] one large volume of UNDW was inhaled, which may cause a more prolonged bronchoconstriction.

We conclude that after inhaling the threshold dose of UNDW a plateau of sustained bronchoconstriction is present between 30 and 180 seconds after inhalation of this dose. This implies that a 5-minute interval between the inhalation of two consecutive doses is long enough to measure the deepest fall in FEV_1.

4.5 UNDW- VERSUS HISTAMINE-INDUCED BRONCHOCONSTRICTION

Introduction

The histamine provocation test is a standardized method for the assessment of bronchial hyperresponsiveness and it is the most frequently used bronchial provocation test in clinical and research lung function laboratories [16,17]. UNDW-induced bronchoconstriction can also be used to measure bronchial hyperresponsiveness [7,9]. In this study we investigated whether the histamine-induced bronchoconstrictor response is correlated to the UNDW-response as assessed with our method of challenging.

Patients and methods

The relation between the UNDW provocation test and the histamine

challenge was investigated in 16 atopic asthmatic patients (Table 4.2) Their mean age was 29.5 $\pm$ 2.2 years and the mean baseline FEV_1 85.9 $\pm$ 4.4% of predicted. The histamine- and UNDW-challenges were performed on 2 different days, at the same time of the day and with an interval of at least one day in between. The baseline FEV_1 on those days was within 10% varation. All medication was stopped for a period of more than eight hours before each test.

UNDW provocation test. The UNDW provocation test was performed as described in paragraph 4.1.

Histamine provocation test. The histamine provocation test was performed according to Ryan et al [18]. The patients inhaled doubling doses of histamine administered with a dosimeter (Jaeger, Würzburg, FRG). The PD_{20}histamine, the dose of inhaled histamine causing a 20% fall in FEV_1 from baseline values, was calculated in μmol histamine from a semi-logarithmic dose-response curve by linear interpolation.

Statistical analysis. Correlations were calculated by the least squares method after $\log_{10}$transformation of the PD_{20} data and expressed as Pearson coefficients. All data are presented as means $\pm$ standard error (SE) and lung function values are expressed as percentage of predicted [19]. Significance was accepted for $p < 0.05$.

Results

The baseline FEV_1 values on the days of measurement were not significantly different. The mean interval between the histamine and UNDW challenge was 3.3 $\pm$ 0.5 days. The PD_{20}histamine was 0.10 $\pm$ 0.03 μmol and the mean PD_{20}UNDW was 2.4 $\pm$ 0.6 ml. A significant correlation existed between the log transformed PD_{20}histamine and PD_{20}UNDW values ($r=0.72$, $p=0.002$), as shown in figure 4.7. There was no correlation between the baseline FEV_1 and the PD_{20}histamine or PD_{20}UNDW.

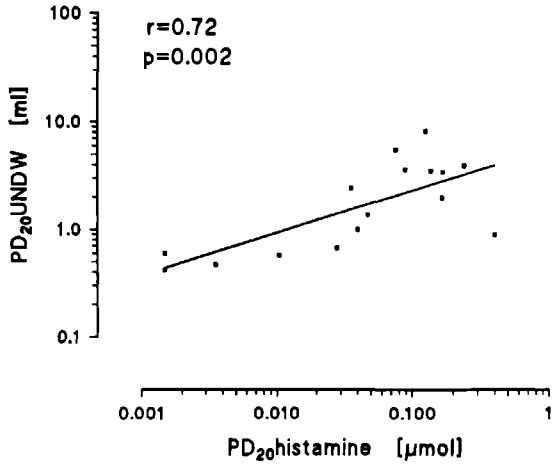

Figure 4.7: Correlation between the UNDW- and histamine-induced bronchoconstrictor response in 16 asthmatic subjects, expressed as the PD_{20}UNDW and the PD_{20}histamine. The r-value indicates the Pearson coefficient of correlation of the PD_{20}-values.

Discussion

In our patients the UNDW provocation test was significantly correlated to the histamine provocation test. Several other investigators have also compared the UNDW provocation test with histamine or methacholine provocation tests, as discussed in paragraph 3.3. In asthmatic children no correlation could be found between the fall in FEV_1 induced by inhalation of methacholine and UNDW [20,21]. In children, who seem to be less susceptible to UNDW bronchoconstriction [21-23], the relationship between responsive-

ness to distilled water and methacholine is probably not comparable with that in adults. In adults other authors have found significant correlations between methacholine- and UNDW- challenge [6,24,25] or histamine and UNDW [26].

We conclude that in adult asthmatic patients bronchial hyperresponsiveness to UNDW and histamine are significantly correlated, although the underlying mechanisms of the bronchoconstriction induced by physical and pharmacological stimuli appear to be different.

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REFRACTORINESS FOR ULTRASONICALLY NEBULIZED DISTILLED WATER AND HISTAMINE AFTER HISTAMINE CHALLENGE

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5.1 ABSTRACT

Refractoriness for bronchial provocation frequently occurs after different challenge tests used to assess bronchial hyperresponsiveness in asthmatic patients. We investigated whether histamine inhalation could cause refractoriness for bronchoconstriction induced by ultrasonically nebulized distilled water (UNDW) and whether histamine causes tachyphylaxis for a subsequent histamine challenge in nine stable asthmatic patients. Preinhalation of histamine induced a significant diminished bronchoconstrictor response to UNDW cumulative dose of inhaled UNDW causing a 20% fall in forced expired volume in 1 s. The mean increased from 3.5 $\pm$ 0.8 ml to 11.8 $\pm$ 2.6 (SE) ml after histamine challenge ($p < 0.01$). However, repeated inhalation of histamine did not change the bronchoconstrictor response to histamine within 1 h after rechallenge ($p > 0.5$). The magnitude of refractoriness for UNDW inhalation after preinhalation of histamine was correlated to the bronchoconstrictor response to histamine ($r = 0.73$, $p < 0.05$). We conclude that inhaled histamine can induce refractoriness for UNDW, which seems to be related to the degree of bronchial hyperresponsiveness.

5.2 INTRODUCTION

Nonspecific bronchial hyperresponsiveness is an important feature of asthma. Pharmacological stimuli like histamine and methacholine [1] and physical stimuli like exercise [2] and ultrasonically nebulized aerosols [3] are frequently used to measure the degree of bronchial hyperresponsiveness in the individual patient and to investigate the underlying mechanisms. Certain asthmatic patients responding to one of these stimuli demonstrate a substantial attenuated response after reexposure to the same stimulus within several hours after the first challenge. This phenomenon, known as tachyphylaxis in farmacological stimuli and refractoriness in physical stimuli, has been demonstrated in most provocation tests [3-8]. The underlying mechanisms are still not clear.

The purpose of this study was to investigate whether refractoriness for ultrasoni-

cally nebulized distilled water after preinhalation of histamine occurs and whether this phenomenon is correlated to tachyphylaxis for repeated inhalation of histamine in asthmatic patients.

5.3 PATIENTS AND METHODS

Subjects. (Table 5.1) Nine stable asthmatic patients participated in the study. Their mean age was 32.3 $\pm$ 3.4 (SE) year and their mean baseline FEV_1 was 83.2 $\pm$ 5.7 % of predicted [9]. Six patients had positive skin tests to common inhalational allergens. All patients were nonsmokers and had no respiratory infection within one month before the study. All patients were treated with inhaled albuterol, and four of them also used beclomethasone. The study protocol was approved by the Ethics Committee of the hospital and all patients gave informed consent before entry into the study.

Table 5.1: Patient characteristics

patient	sex	age (y)	FEV_1 (% pred)	atopic	PD_{20}hist (μg)(*)	medication (**)
1.	M	50	60.5	-	7.7	s, b
2.	M	41	57.7	-	29.3	s, b
3.	M	35	80.7	+	44.1	s, b
4.	F	21	100.6	+	76.5	s, b
5.	F	26	75.2	+	94.1	s
6.	F	24	84.5	+	99.9	s
7.	M	25	91.9	+	279.0	s
8.	F	39	95 9	-	454.5	s
9.	F	30	101.6	+	545.9	s
mean		32.2	83.6		171.4	
SE		3.4	5.7		65.0	

(*) PD_{20}Hist: The dose of inhaled histamine causing a 20% fall in FEV_1; 1 μg histamine = 0.0033 μmol.
(**) s: salbutamol, b: beclomethasone dipropionate

Study Design. Each subject was studied on 4 different days with an interval of at least one day. All medication was stopped 8 hours before each test. On days 1 and 3, UNDW provocation tests were performed. On day 2, a histamine provocation test was followed by an UNDW test when the FEV_1 had returned spontaneously to within 10% of the baseline values. On day 4, a histamine provocation test was followed by a second histamine provocation test when the FEV_1 had again returned spontaneously within 10% of the baseline values.

UNDW provocation test. The UNDW test was performed according to Anderson et al [3]. An ultrasonic nebulizer (Ultraneb 99, DeVilbiss, Somerset, PA) was used at a fixed output of 2.00 $\pm$ 0.10 ml/min. The patient inhaled the aerosol at tidal breathing through a mouthpiece with tightened lips and the nose clipped. A two-way valve (Leardal IV, Stavanger) was placed between the aerosol hose and the mouthpiece. A Wright respirometer (British Oxygen, London, UK) was attached to the two-way valve to measure the total volume of inhaled air. The patient started with inhaling 20 liters of ambient air through the system. Thereafter, 3, 5, 10, 20, 40, 80, and 160 liters of air with UNDW were successively inhaled at 5-min intervals. Lung function was measured by recording flow-volume curves obtained with a pneumoscreen (model II, Jaeger, Wurzburg, FRG) at 30, 90, and 120 s after inhalation of each dose. The test was stopped when a 20% fall in FEV_1 was achieved or when the last dose of air with UNDW,i.e. 160 liters, was inhaled. Before and after each test, the nebulizer chamber and aerosol hose were weighed. A log dose-response curve was constructed, and the cumulative dose of inhaled distilled water (in ml H_2O) causing a 20% fall in FEV_1 compared with the lowest post-air FEV_1 (PD_{20}UNDW) was calculated by linear interpolation.

Histamine provocation test. The histamine provocation test was performed according to Ryan et al [10]. The patient inhaled doubling doses of histamine, increasing from 1.35 μg to 720 μg, administered with a dosimeter (Jaeger, Wurzburg, FRG). The PD_{20}histamine, the dose of inhaled histamine causing a 20% fall in FEV_1, was calculated in micrograms by linear interpolation. The total dose of histamine inhaled was also calculated.

Statistical analysis. The PD_{20}UNDW values obtained at day 1 and 3 were averaged for comparison with the PD_{20}UNDW values after prior inhalation of histamine.

The PD_{20}histamine data of day 2 and 4 were also averaged for comparison with the second PD_{20}histamine on day 4. Before statistical analysis a natural log transformation of PD_{20}UNDW and PD_{20}histamine values was performed. An analysis of variance for repeated measurements was performed on data where a student-t test for correlated samples was not appropriate. Correlations were calculated with the Spearman rank method on non-log transformed data. All data are presented as means $\pm$ SE and statistical significance was accepted at $p < 0.05$ for all tests.

5.4 RESULTS

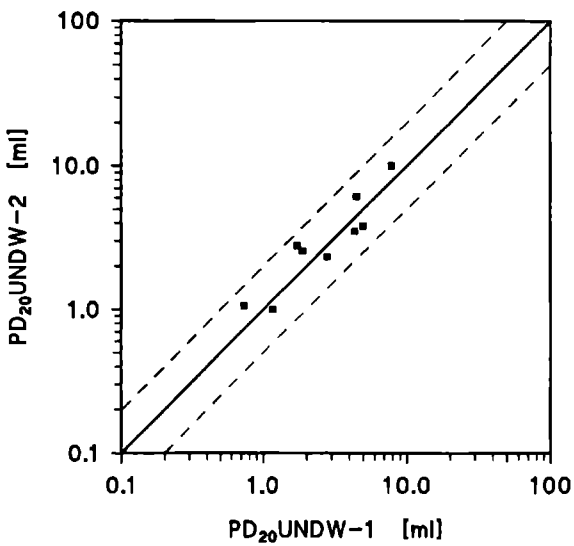

Figure 5.1: Reproducibility of the UNDW tests. The PD_{20}UNDWs on day 1 and day 3 (PD_{20}UNDW (1) and PD_{20}UNDW (2), respectively) were within 1 doubling dose.

The baseline FEV_1 before each test on the 4 study days was within 10% variation with mean values ranging from 2.99 $\pm$ 0.27 L to 3.08 $\pm$ 0.33 L. The reproducibility of PD_{20}UNDW on day 1 and 3 is shown in figure 5.1. The differences between each PD_{20}UNDW on day 1 and 3 were within one doubling dose and the mean PD_{20}UNDW's on those two days were not significantly different. Figure 5.2 shows the log dose-response curves of the ultrasonically nebulized distilled water provocation tests for all subjects individually.

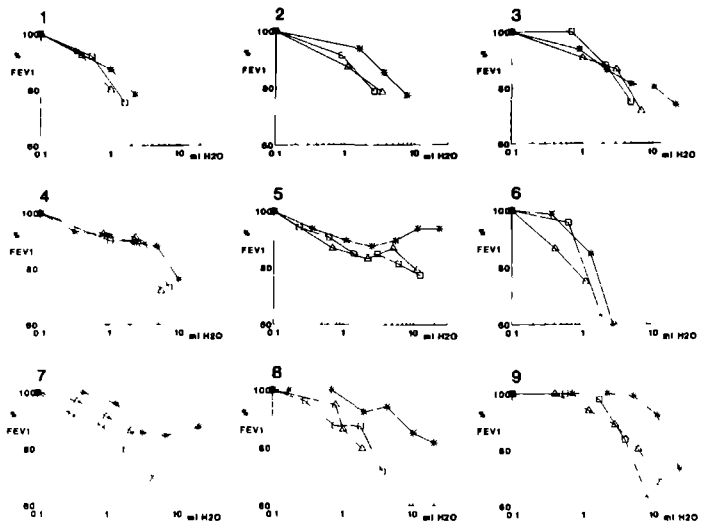

*Figure 5.2: Log dose response curves of UNDW provocation tests in 9 asthmmatic patients. ■ : UNDW provocation test on day 1, ▲: UNDW provocation test on day 3, *: UNDW after prior inhalation of histamine on day 2*

There was no significant difference between between the distilled water provocation tests on day 1 and 3, with mean PD_{20} values of 3.3 $\pm$ 0.8 and 3.7 $\pm$ 1.0 ml H_2O, respectively. Three of nine patients (patients 5, 7 and 8) did not reach a 20% fall in FEV_1 after

inhalation of the last dose of distilled water after pre-inhalation of histamine. They inhaled 20 ml of distilled water cumulatively. In all subjects the UNDW and histamine challenge after pre-inhalation of histamine were performed within one hour after the end of the baseline histamine inhalation test. After preinhalation of histamine the mean PD_{20}UNDW significantly increased to 11.8 $\pm$ 2.6 ml H_2O ($p < 0.01$), as shown in figure 5.3a. The increase in mean PD_{20}UNDW was 1.5 $\pm$ 0.3 doubling dose.

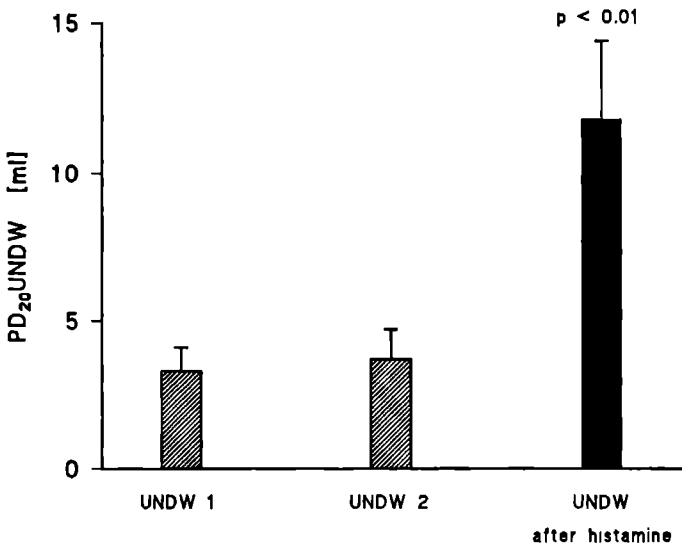

Figure 5.3a: Mean PD_{20}UNDW before (UNDW 1 and UNDW 2) and after histamine challenge. A significant increase in mean PD_{20}UNDW ($p < 0.01$) was induced by preinhalation of histamine.

The PD_{20}histamine measured on day 2 and 4 were identical, with mean values of 171.4 $\pm$ 65.0 μg and 172.9 $\pm$ 62.7 μg, respectively. After preinhalation of histamine on day 4 a mean PD_{20}histamine of 205.8 $\pm$ 86.6 μg was measured for the second challenge, which value was not significantly different from the baseline PD_{20}histamine values obtained at day 2 and 4 (Figure 5.3b).

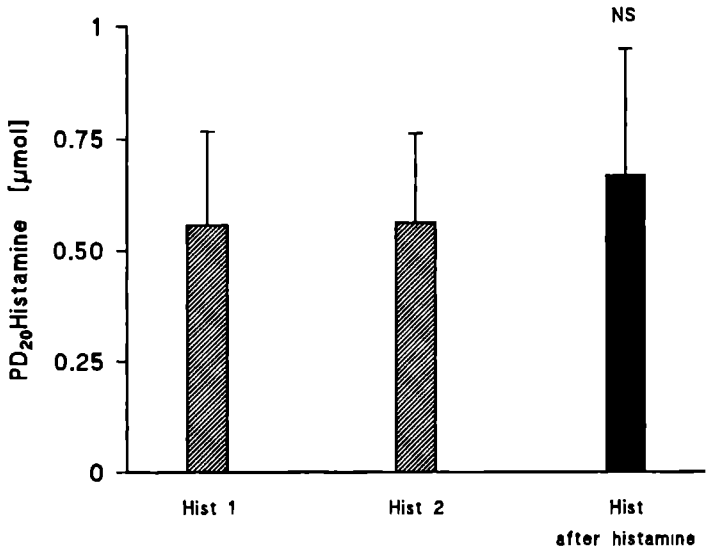

Figure 5.3b: Mean baseline PD_{20}histamine values measured on two study days (Hist 1 and Hist 2) and the mean PD_{20}histamine obtained after histamine challenge. There is no significant difference in mean PD_{20}histamine values ($p > 0.5$).

The difference between the baseline PD_{20}UNDW measured on day 1 and 3 and the PD_{20}UNDW after pre-inhalation of histamine was calculated and called the shift in PD_{20}UNDW. A significant correlation was found between the shift in PD_{20}UNDW and the baseline PD_{20}histamine ($r= 0.73$, $p= 0.036$). Also, the last dose of histamine inhaled, before the UNDW test after preinhalation of histamine was started on day 2, was correlated to the change in PD_{20}UNDW ($r= 0.72$, $p=0.04$). No correlation was found between the change in PD_{20}UNDW and the total dose of histamine inhaled.

5.5 DISCUSSION

In this study we have demonstrated that preinhalation of histamine before inhalation of UNDW causes refractoriness for UNDW-induced bronchoconstriction in a

group of mildly asthmatic patients. However, prior inhalation of histamine did not change the bronchoconstrictor effect of a second histamine challenge within one hour on the same day.

Refractoriness to the bronchoconstrictor effect of the same stimulus has been shown for exercise [7], inhalation of hypertonic and hypotonic aerosols [3,5], and inhalation of adenosine 5'monophosphate [6]. Refractoriness after hyperventilation-induced bronchoconstriction has not clearly been demonstrated [11]. Tachyphylaxis has been reported to occur after repeated inhalation of histamine in asthmatic subjects [8,12].

The underlying mechanism of refractoriness is still unknown. Cromoglycate, a mast-cell-stabilizing agent, can inhibit UNDW-induced bronchoconstriction [3]. Significant neutrophil chemotactic activity levels in blood have been found after UNDW provocation [13]. Also, leukotrienes D_4 and E_4 seems to be involved because pretreatment with an antagonist of these mediators diminished UNDW-induced bronchoconstriction [14]. Depletion of bronchoconstrictor mediators from mast cells has been suggested to have a role in the refractoriness for repetitive UNDW provocation [3]. In histamine provocation, pretreatment with cromoglycate could not prevent bronchoconstriction [15], so mast-cell-derived mediators seem to have no important role in the histamine-induced bronchoconstrictor response. Therefor mast cell depletion seems to be unlikely to be the cause in histamine-induced refractoriness for UNDW.

Bronchodilating cyclooxygenase products like prostaglandin E_2 seem to have an important role in refractoriness and tachyphylaxis [8,16]. Pretreatment with indomethacin inhibited both refractoriness for repeated inhalation of UNDW and tachyphylaxis for repeated inhalation of histamine [8,16]. Blockade of the H_2-receptors with cimetidin, a specific H_2-receptor antagonist, inhibited tachyphylaxis for repeated inhalation of histamine [17]. Jackson et al. [17], therefore postulated that H_2-receptors modulate prostaglandin release after histamine challenge and that these receptors seems to be involved in tachyphylaxis. Prostaglandin release due to histamine provocation probably has some inhibiting effects on mediator release in UNDW challenge.

We also investigated whether in asthmatic patients a correlation can excist between the total amount of histamine inhaled and the magnitude of refractoriness for UNDW after preinhalation of histamine. No such correlation was found, but we did find

a significant correlation the last dose of inhaled histamine and the shift in PD_{20}UNDW. A significant correlation was also found between the PD_{20}histamine and the shift in PD_{20}UNDW after preinhalation of histamine. These correlations suggest that the magnitude of refractorines is not determined by the total amount of inhaled histamine, but that the severity of the bronchial hyperresponsiveness appears to be more important. Thus patients with severe bronchial hyperresponsiveness to histamine are less susceptable for refractoriness for UNDW-induced bronchoconstriction than asthmatic subjects with a lower degree of bronchial hyperresponsiveness.

Repeated inhalations of histamine did not cause a significant shift in PD_{20}histamine. Although there was a small rise in mean PD_{20}histamine after preinhalation of histamine the shift did not reach significance. These results comfirm those of Ruffin et al. [18], who also could not demonstrate a difference in PC_{20}histamine after repeated inhalation of histamine. Even four successive histamine provocation tests on one day could not induce tachyphylaxis in their group of asthmatic patients. Schoeffel et al. [19] were also not able to induce tachyphylaxis with three succesive inhalation challenges with histamine, although these autors did not calculate changes in PD_{20}histamine. Kung et al. [20] could also not demonstrate changes in PD_{20}histamine after consecutive histamine challenges. These data and the results of our study are in contrast with those of Jackson et al [17] and Connolly et al [12], who showed significant tachyphylaxis for histamine after repeated inhalation in their groups of patients, although the shift in mean PC_{20}histamine [17] and mean PD_{20}histamine [12] were within one doubling dose. The time between the first test and the rechallenge was the same for all these studies, 30 - 90 min.

An explanation for the differences between these studies may be a difference in patients selection. Ruffin et al. [18] investigated subjects with mild to moderate asthma, with a mean PC_{20}histamine of 2.78 mg/ml (range: 0.3 - 5.3 mg/ml), and only one of their 12 patients was atopic. Jackson et al. [17] investigated a group of mild atopic asthmatics, mean PC_{20}histamine 3.01 mg/ml (range: 1.5 - 5.0 mg/ml). Our group was a mixed population of mild to moderate asthmatics with a mean PD_{20}histamine values of 171.9 μg (3.83 mg/ml, range: 0.15 - 11.1 mg/ml) and 6 patients were atopic. Connelly et al.[12] investigated a group of 20 patients and 18 were atopic, and Kung et al.[20] investigated a group of asymptomatic asthmatics and did not differentiate between atopic and nonatopic

patients. The PD_{20}histamine used in the last two studies [15,18] was calculated as the cumulative dose of inhaled histamine, and therefore can not be compared with the values mentioned above.

Mild stable asthma which does not require inhaled corticosteroids, has been suggested to be a discriminating aspect for the occurence of tachyphylaxis for histamine [8]. However, in a group of asymptomatic patients [20] who did not use any medication, tachyphylaxis could not be demonstrated. Thus there is no clear difference between the study groups which can explain the differences in outcome other than the atopic status; however, distinction between atopic and nonatopic only with skintests seems to be of less importance [21] and is limited to the panel of inhalation allergens used.

We conclude that preinhalation of histamine causes refractoriness for inhalation of UNDW in asthmatic patients. The magnitude of this refractoriness seems to be inversely correlated to the severity of bronchial hyperresponsiveness to histamine. Tachyphylaxis for histamine could not be demonstrated in these patients.

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THE PROTECTIVE EFFECTS OF IPRATROPIUM BROMIDE AND TERBUTALINE ON DISTILLED WATER-INDUCED BRONCHOCONSTRICTION.

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submitted for publication

6.1 ABSTRACT

UNDW-induced bronchoconstriction can be inhibited by β_2-agonists, drugs with mast cell stabilizing properties like cromoglycate, and anticholinergic drugs like atropine. The dose related protective effects of ipratropium bromide in UNDW challenge are not clear.

In a randomized, double-blind, placebo-controlled study, we investigated the protective effects of ipratropium bromide 160 μg and 320 μg and terbutaline 500 μg on UNDW-induced bronchoconstriction in nine stable asthmatic patients. To compare the drug effects, we determined the PD_{20}UNDW and the reactivity and sensitivity of the UNDW dose-response curve. Both drugs caused a significant increase ($p<0.001$) in baseline FEV_1 with no significant differences between the drugs or both doses of ipratropium bromide. Pre-inhalation of ipratropium bromide 320 μg and terbutaline 500 μg inhibited UNDW-induced bronchoconstriction as measured by PD_{20}UNDW and sensitivity ($p<0.01$), whereas ipratropium bromide 160 μg had no protective effect. There was no correlation between the increase in baseline FEV_1 and PD_{20}UNDW indicating that the protective effect on UNDW-induced bronchoconstriction is not dependent on the bronchodilatation induced by terbutaline and ipratropium bromide. It also appears that the UNDW-induced bronchoconstriction is at least partly vagally mediated.

6.2 INTRODUCTION

Bronchial hyperresponsiveness is a major characteristic feature of bronchial asthma[1]. Inhalation of ultrasonically nebulized distilled water (UNDW) can induce bronchoconstriction in asthmatic subjects and has been used for assessment of bronchial hyperresponsiveness[2]. The underlying mechanism of UNDW-induced bronchoconstriction has not been elucidated yet.

Acknowledgement: We kindly thank Astra Pharmaceutics and Boehringer Ingelheim for providing the test medication.

Pre-inhalation of sodium cromoglycate[2] and nedocromil sodium[3] can inhibit UNDW-induced bronchoconstriction, indicating that mast cell-derived mediators probably are involved. Furthermore, the cholinergic nervous system seems to be involved, since pre-inhalation of atropine can prevent UNDW-induced bronchoconstriction[4]. The protective effects of the non-selective muscarine receptor antagonist
ipratropium bromide on UNDW-induced bronchoconstriction is not clear yet. Doses used in general practice, i.e. 40 μg and 80 μg, did not show any protection[5,6]. β_2-agonists, like salbutamol[5] and fenoterol[7] on the contrary can totally block the UNDW-induced bronchoconstrictor response.

Therefore, the aim of this study was to investigate the effect of ipratropium bromide on UNDW-induced bronchoconstriction. We used two different doses of ipratropium bromide to assess whether its effect is dose-dependent and we compared the effects of ipratropium bromide with those of placebo and the β_2-agonist terbutaline.

6.3 PATIENTS AND METHODS

Table 6.1: patient characteristics

patient	sex	age (y)	FEV_1 (% pred.)	PD_{20}hist (μmol)	PD_{20}UNDW (ml)	medication (*)
1	F	24	90.9	0.20	6.5	a, b
2	F	33	118.3	0.18	1.4	a, b
3	M	53	56.8	0.03	1.3	a, b
4	M	50	61.4	nd	2.0	a, b
5	F	22	73.9	0.01	5.3	a
6	F	44	84.3	0.23	8.1	a
7	M	37	54.1	0.002	1.3	a, b, ib
8	F	43	108.9	0.23	4.0	a, b
9	M	16	92.5	0.05	3.0	a, b
mean		38.9	82.3	0.12	3.6	
SE			4.3	0.04	0.8	

(*) a: salbutamol, b: beclomethasone, ib: ipratropium bromide, nd: not done

Subjects. Nine stable asthmatic subjects participated in the study, their characteristics are given in table 6.1.

All patients, except for patient no. 9, were non-allergic with respect to history and negative reactions to intracutaneous skin tests. Inhalation of a $ß_2$-agonist induced an increase in FEV_1 of more than 15% and all patients reacted to inhalation of UNDW before the start of the trial with at least a 20% decrease in FEV_1. Anti-asthmatic medication was stopped for a period of 8 hours before each test, but inhaled corticosteroids were continued without changing the dose during the study. None of the patients had used systemic corticosteroids for a period of at least three months or suffered from a respiratory tract infection for a period of at least one month before the start of the study. The study was approved by the local Ethics Committee and all patients gave their written informed consent.

Study design. The patients attended the lung function laboratory on four different days at the same time of the day with intervals of at least one day. The baseline FEV_1 on those days had to be within 10% variation. After recording baseline flow-volume curves (Pneumoscreen II, Jaeger, Würzburg, FRG) the subjects inhaled double-blind and in a randomized order: placebo, ipratropium bromide 160 μg, ipratropium bromide 320 μg or terbutaline 500 μg. Ipratropium bromide was inhaled by means of a metered dose inhaler, 20 μg per puff, through a 750 ml spacer device and terbutaline was inhaled as a powder by means of a turbuhaler® (Astra, Lund, Sweden), 500 μg per inhalation. Thirty minutes after inhalation of the test drugs an UNDW provocation test was performed.

Measurements. Flow-volume curves were recorded to measure lung function before and during the UNDW provocation tests.

UNDW provocation tests were performed with the Ultraneb 99 ultrasonic nebulizer (DeVilbiss, Somerset, USA), according to a modified method described in paragraph 4.1. The PD_{20}UNDW, the cumulative dose of UNDW causing a 20% fall in FEV_1, was calculated from post-air values by linear interpolation on a semi-logarithmic curve and expressed in ml H_2O[a]. If a 20 % fall in FEV_1 was not achieved, the PD_{20}UNDW was equated to the total amount of inhaled UNDW.

To assess the effect of ipratropium bromide and terbutaline on the dose-response curve,

the sensitivity, the slope of a linear regression line of the total dose-reponse curve[9] and reactivity, the slope of a linear regression line of the steepest part of the curve[10], were calculated, as shown in figure 6.1.

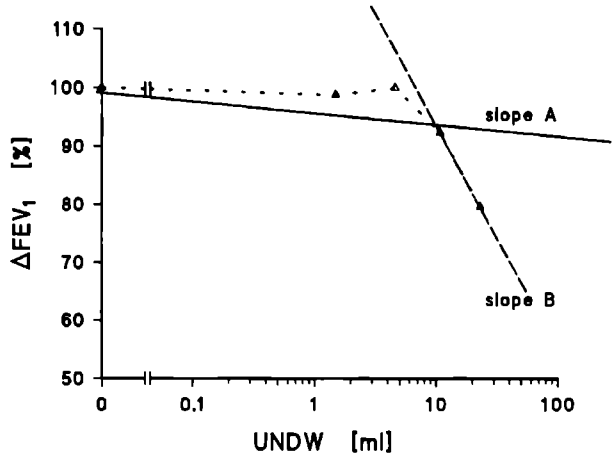

Figure 6.1: A dose-response curve, that of patient nr.8, with on the X-axis the amount of inhaled UNDW, and on the Y-axis the FEV_1 as percentage of the post-air value. The broken line represents the dose-response curve. Slope A, the linear regression of the total curve, expresses the sensitivity and slope B, a linear regression of the steepest part of the curve, expresses the reactivity.

Statistical Analysis. The FEV_1 is expressed as percentage of predicted[11]. The increase in FEV_1, 30 minutes after inhalation of the drugs, is expressed as percentage of the baseline FEV_1. The changes in PD_{20}UNDW are expressed in doubling doses calculated from placebo values. All data were analyzed by the Wilcoxon test and multiple comparison was performed with the Bonferroni correction. Correlations were calculated by the Spearman-rank test. All data are presented as means with standard error (± SE). Statistical significance was accepted for $p < 0.05$.

6.4 RESULTS

The baseline FEV_1 values on the 4 study days were not significantly

different (p=0.6). The increase in FEV_1 versus the increase in $PD_{20}UNDW$, expressed in doubling doses, both calculated from baseline values is shown in figure 6.2.

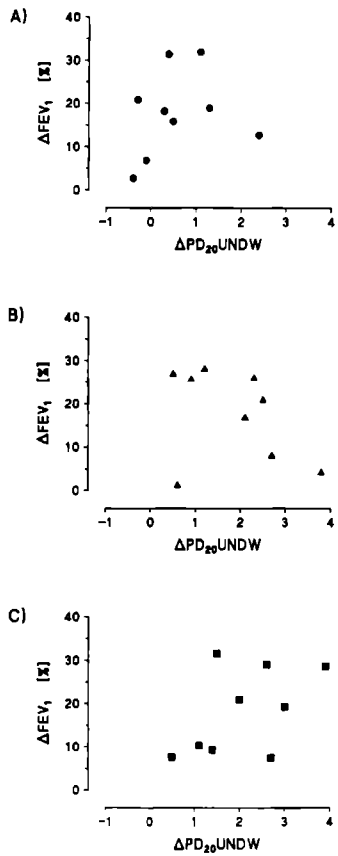

Figure 6.2: A scattergram representing the increase in FEV_1 versus the increase in $PD_{20}UNDW$, calculated from baseline values, after inhaled ipratropium bromide 160 μg (panel A), ipratropium bromide 320 μg (panel B) and terbutaline (panel C).

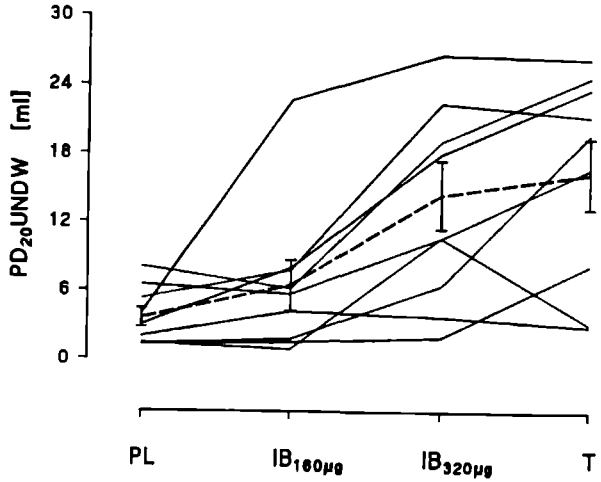

Figure 6.3: The changes in UNDW-induced bronchoconstriction expressed as PD_{20}UNDW after preinhalation of placebo (PL), ipatropium bromide 160 µg ($IB_{160\ \mu g}$) and 320 µg ($IB_{320\ \mu g}$), and terbutaline 500 µg (T). Solid lines indicate individual changes in PD_{20}UNDW. The broken line represents the mean changes in PD_{20}UNDW (± SE).

Table 6.2: Effects of placebo, ipratropium bromide 160 µg and 320 µg and terbutaline 500 µg on the mean values (± SE) of PD_{20}UNDW (expressed in ml H_2O), and reactivity and sensitivity of the UNDW dose-response curves (both expressed in arbitrary units).

	PD_{20}UNDW	REACTIVITY ($slope_A$)	SENSITIVITY ($slope_B$)	CORRELATION PD_{20}-$slope_A$	CORRELATION PD_{20}-$slope_B$
Placebo	3.6 ± 0.8	-40.7 ± 10.0	-7.3 ± 1.9	NS	r_s=0.99 p=0.005
Ipratropium Bromide 160 µg	6.5 ± 2.2	-29.8 ± 4.9	-5.0 ± 1.3	NS	r_s=0.98 p=0.006
Ipratropium Bromide 320 µg	14.5 ± 3.0*♦	-22.5 ± 5.6	-2.0 ± 0.7*♦	NS	r_s=0.98 p=0.006
terbutaline 500 µg	16.4 ± 3.1*♦	-18.6 ± 6.1	-1.6 ± 0.6*♦	NS	r_s=0.95 p=0.007

** : $p < 0.005$ versus placebo*
♦ : $p < 0.0125$ versus ipratropium bromide 160 µg

The mean baseline FEV_1, increased from 79.1 $\pm$ 6.9% to 80.9 $\pm$ 8.3 % after placebo (p=0.45), from 79.9 $\pm$ 7.2 % to 92.7 $\pm$ 6.5 % after ipratropium bromide 160 μg (p=0.008), from 82.8 $\pm$ 7.0 % to 95.5 $\pm$ 6.0 % after ipratropium bromide 320 μg (p=0.008) and from 79.6 $\pm$ 7.5 to 91.8 $\pm$ 7.8 % after terbutaline (p=0.008). The two doses of ipratropium bromide as well as terbutaline induced a similar, more than 15% increase in baseline FEV_1.

The individual and mean changes in PD_{20}UNDW are shown in table 6.2 and figure 6.3. Ipratropium bromide 160 μg improved the PD_{20}UNDW 0.6 $\pm$ 0.3 doubling dose, which was not significantly different from placebo (p=0.17). Ipratropium bromide 320 μg and terbutaline provided a significant protection against UNDW-induced bronchoconstriction compared to placebo and increased the PD_{20}UNDW 1.9 $\pm$ 0.4 and 2.1 $\pm$ 0.4 doubling dose respectively (p=0.002). This protection was significantly better than that of ipratropium bromide 160 μg (p=0.01), but there was no significant difference in protection between ipratropium bromide 320 μg and terbutaline 500 μg (p=0.38). Terbutaline caused a total inhibition of the UNDW-induced bronchoconstrictor response in 3 patients, with a mean fall in FEV_1 of 2.9 $\pm$ 1.5 %, whereas ipratropium bromide 320 μg completely inhibited the UNDW response in 2 patients. No significant correlation was found between the increases in FEV_1 30 minutes after inhalation and the changes in PD_{20}UNDW induced by the drugs studied.

The reactivity of the dose-response curves was not significantly changed by inhalation of the test drugs in comparison with placebo (p=0.43), as shown in table 6.2. The sensitivity of the dose-response curves, was not significantly different after pretreatment with ipratropium bromide 160 μg compared to placebo (p=0.13). Ipratropium bromide 320 μg and terbutaline induced a significant change in sensitivity of the dose-response curve compared to placebo (p=0.003) and ipratropium bromide 160 μg (p=0.006). There was no significant difference between the sensitivity of the UNDW dose-respons curves after the inhalation of ipratropium bromide 320 μg or terbutaline (p=0.68). The sensitivity of the dose-response curve was strongly correlated to the PD_{20}UNDW values (table 6.2). There were no significant correlations between the PD_{20} values and the reactivitie of the UNDW dose-response curves.

6.5 DISCUSSION

In this study we have demonstrated that pre-inhalation of ipratropium bromide 320 μg or terbutaline 500 μg can inhibit UNDW-induced bronchoconstriction in asthmatic patients, although the bronchoconstrictor response was not totally blocked in all patients. The bronchodilatation induced by the study drugs was not maximal for all patients as shown by the mean FEV_1 30 minutes after inhalation, ranging from 91.8 to 95.5 % of the predicted values. Preinhalation of ipratropium bromide 160 μg increased the baseline FEV_1 significantly and to the same degree as ipratropium bromide 320 μg and terbutaline 500 μg. However, ipratropium bromide 160μg did not inhibit UNDW-induced bronchoconstriction as measured by the PD_{20}UNDW and the sensitivity of the dose-response curve.

We did not find a correlation between the increase in FEV_1 and the degree of protection as illustrated in figure 6.2, which indicates that the protective effect was not solely due to the bronchodilator response of the drugs. This means that the protective effect of ipratropium bromide is related to the amount of inhaled drug. These findings are supported by the results of other studies [5,12]. Doses of 80 μg inhaled ipratropium bromide had no protective effect, whereas doses above 200 μg induced a significant protection against UNDW-induced bronchoconstriction. The mechanism of the inhibition by ipratropium bromide of the UNDW-induced bronchoconstriction in asthmatics is not known. In contrast to $ß_2$-agonists, ipratropium bromide has no stabilizing effects on mast cell degranulation as shown during allergen provocation[13]. Decreased airway smooth muscle supersensitivity[14] or an inhibition of a vagal reflex induced by ipratropium bromide[15], might be the mode of action of this drug. Since the protective effect of muscarinic receptor antagonists to bronchoconstrictor stimuli only appears to be mediated through the inhibition of acetylcholine release[16], our results support the idea that bronchoconstriction induced by UNDW in asthmatics is at least partially mediated by a vagal reflex mechanism.

Our data show a large individual variation in the protective effect of ipratropium bromide 320 μg on UNDW-induced bronchoconstriction. This finding is confirmed by

Ihre et al.[17], who found a remarkable interindividual variation in bronchodilation and protection for histamine-induced bronchoconstriction due to ipratropium bromide, whereas they found only a small intraindividual varation.

The protective effect of terbutaline was not complete in all subjects. This was not what we had expected from literature, since $ß_2$-agonists like salbutamol totally blocked UNDW-induced bronchoconstriction[5]. Terbutaline 500 μg, however, showed significantly less protection in histamine-induced bronchonstriction than fenoterol 400 μg and salbutamol 200 μg[18], which may support our findings.

Intraindividually the PD_{20}UNDW is a well reproducible threshold when dose-response curves are compared[8]. However, there may be some limitations of this threshold in dose-response curves where a 20% fall in FEV_1 after challenge is not achieved, as occurred in some patients in this study after pretreatment with a bronchodilator. For these patients the PD_{20}UNDW was equated to the total amount of inhaled UNDW. Moreover, this threshold gives no information about the shape of the dose-response curve. We therefore calculated the reactivity and sensitivity of the individual dose-response curves after placebo and both study drugs, which as far as we know, has not been done before for the UNDW-induced bronchoconstrictor response. The sensitivity was strongly correlated with the PD_{20}UNDW and the changes in the sensitivity of the dose-response curves induced by ipratropium bromide and terbutaline were comparable to those in PD_{20}UNDW. The reactivity, however, showed no significant change after prior bronchodilatation, although the data seem to indicate a trend towards a less steep slope after inhalation of the bronchodilator drugs. The large standard error due to the large interindividual variation of the reactivity in this small group of patients, may add to non significant changes and shows the limitation of this parameter.

We conclude that in comparison with a regular dose of terbutaline only a high dose of inhaled ipratropium bromide provides significant protection against UNDW-induced bronchoconstriction. This inhibition is not solely related to the bronchodilator effect of ipratropium, but is probably also due to blockade of a vagally mediated reflex induced by UNDW.

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EFFECTS OF INHALED BECLOMETHASONE AND NEDOCROMIL SODIUM ON BRONCHIAL HYPERRESPONSIVENESS TO HISTAMINE AND DISTILLED WATER

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7.1 ABSTRACT

In a randomized crossover study we compared the effects of inhaled nedocromil sodium, 4 mg q.i.d., with inhaled beclomethasone dipropionate, 200 μg q.i.d. in 23 atopic asthmatic patients. After a 3-week single-blind placebo period, regarded as the baseline, and after 4 and 8 weeks of active treatment, drug effects were assessed with regard to bronchial hyperresponsiveness to histamine and distilled water, lung function, and β_2-agonist use. After 4 and 8 weeks of treatment, nedocromil sodium decreased the histamine responsiveness ($p<0.005$ and $p<0.0005$), but not the distilled water responsiveness, and did not improve lung function and peak flow measurements compared to the baseline. After 4 and 8 weeks of treatment, beclomethasone caused a significant increase in lung function ($p<0.005$) and decrease in bronchial hyperresponsiveness to histamine ($p<0.0005$) and distilled water ($p<0.0005$) as compared to the baseline. β_2-agonist use was significantly diminished after an 8-week treatment with beclomethasone, whereas nedocromil sodium had no effect. Treatment with beclomethasone was superior to treatment with nedocromil sodium with regard to bronchial hyperresponsiveness to histamine and distilled water ($p<0.0005$ and $p<0.005$), lung function ($p=0.003$), peakplow measurements ($p<0.05$) and β_2-agonist use ($p<0.005$).

7.2 INTRODUCTION

Bronchial hyperresponsiveness to a variety of chemical, physical and pharmacological stimuli is one of the major characteristics of bronchial asthma [1,2]. The underlying mechanism of bronchial hyperresponsiveness is still unknown, but several aspects have been elucidated recently. Disruption of the epithelial layer, inflammatory changes in the airway wall and possibly an imbalance in the autonomic regulation of the airway appear to contribute to the pathophysiology of bronchial hyperresponsiveness [1,3].

The presence and the degree of bronchial hyperresponsiveness can be assessed by bronchoprovocation tests with pharmacological and physical stimuli. Hargreave et al. [4] found a correlation between the severity of asthma and the degree of bronchial hyperres-

ponsiveness to histamine and methacholine. Non-specific stimuli like exercise [5] and ultrasonically nebulized distilled water (UNDW) [6] can also be used for the assessment of bronchial hyperresponsiveness. The latter challenges are supposed to have the advantage of corresponding better with the daily exposure of the asthmatic subject to non-specific stimuli [7].

The treatment of asthma is focussed on diminishing the inflammatory process and the bronchial hyperresponsiveness [8]. In a previous study we compared the effects of sodium cromoglycate and budesonide on bronchial hyperresponsiveness in asthmatic subjects [9]. Budesonide induced a significant decrease in bronchial hyperresponsiveness to histamine and exercise, whereas sodium cromoglycate did not have such effects. Nedocromil sodium, a pyranoquinoline dicarboxylic acid derivative, has anti-inflammatory properties, as demonstrated by in vitro and in vivo experiments, and seems to be more potent than sodium cromoglycate [10]. Nedocromil sodium can inhibit early- and late-phase asthmatic responses after allergen inhalation [11], and prevents bronchoconstriction induced by inhaled SO_2 [12], cold air [13], distilled water [14], substance P [15], adenosine [16], and exercise [17]. However, the place of this drug in the treatment of asthma has not been defined yet. The aim of the present study was to investigate the effects of regularly inhaled nedocromil sodium in comparison with inhaled beclomethasone diproprionate on lung function, bronchial hyperresponsiveness to histamine and distilled water, and β_2-agonist use in allergic asthmatic subjects.

7.3 PATIENTS AND METHODS

Subjects. Twenty-eight non-smoking subjects with allergic bronchial asthma [18] participated in the study. All patients were recruited from the hospital outpatient department. Some characteristics of these patients are shown in table 7.1.

Allergy was defined as two or more positive intracutaneous skin test reactions to common airborne allergens. Patients with seasonal allergy did not participate in the study during that specific season. The pre-challenge forced expiratory volume in one second (FEV_1) had to be $\geq$ 50% of the predicted values [19] and reversibility of FEV_1 had to be $\geq$ 15% in

Table 7.1: Patient characteristics.

patient	sex	age (y)	FEV_1 (%pred)	MEF_{50} (%pred)	MEF_{25} (%pred)	PD_{20} hist (μmol)	PD_{20} UNDW (ml)	previous medication
1	F	40	100	72	78	0.05	1.4	s, b
2	F	35	85	49	41	0.05	1.0	s, b
3	F	27	68	34	39	0.50	13.7	s
4	M	26	89	74	64	0.40	0.9	s, b
5	M	22	95	110	155	0.24	10.7	s
6	F	23	87	75	53	0.09	0.6	s, b
7	F	38	82	37	37	0.12	8.2	s, b
8	M	25	51	23	23	0.002	0.6	s, c
9	M	26	89	53	61	0.09	3.6	s, b
10	F	40	72	50	62	0.19	5.3	s, b
11	M	26	101	73	63	0.24	6.2	s, b
12	M	21	63	31	18	0.002	0.1	s, bud
13	F	44	99	76	66	0.01	1.7	s, b
14	M	38	66	36	33	0.24	4.3	s, bud
15	F	32	74	56	64	0.002	0.4	s, b
16	M	17	87	55	51	0.03	9.8	s, b
17	F	19	91	68	46	0.17	2.4	s, bud
18	M	28	89	52	57	0.16	2.0	s, bud
19	F	38	98	99	83	0.03	0.7	s, b
20	F	21	100	58	54	0.004	0.5	s, b
21	M	31	71	38	42	0.17	3.4	s, bud
22	M	19	78	46	40	0.002	0.3	s, b
23	M	27	107	93	81	0.09	-	s
24	F	50	93	66	77	0.04	2.4	s, b
25	M	38	65	34	34	0.04	5.5	s, b
26	M	21	82	50	41	0.06	1.6	s, b
27	M	17	70	40	39	0.04	1.0	s, b
28	M	30	103	59	48	0.24	3.5	s
mean		29.3	84.1	57.3	55.3	0.12	3.4	
SE		1.7	2.7	4.0	4.9	0.02	0.7	

FEV_1 : forced expiratory volume in one second; MEF_{50} and MEF_{25} : maximal expiratory flow when 50% and 25% of the forced vital capacity have to be expired; s = salbutamol powder inhalation; c = cromoglycate powder inhalation; b = beclomethasone powder inhalation; bud = budesonide aerosol.

response to an inhaled β_2-agonist.

The PD_{20}histamine, the dose of histamine causing a 20% fall in FEV_1 from pre-challenge values, was below 0.59 μmol (<4 mg/ml) inhaled histamine for all subjects. None of the patients had used systemic corticosteroids for a period of six months or had suffered from had a respiratory tract infection for a period of one month before the start of the study. Twenty-three patients used inhaled corticosteroids before entering the study with an

average dose of 400 μg twice a day to control their asthma. All previous medication was stopped when the patients entered the first placebo period.

The study was approved by the local Ethics Committee and all patients gave their written informed consent.

Study Design. The study was carried out according to a randomized crossover and double-dummy design (figure 7.1). A three-week single-blind wash-out placebo period was followed by two periods of double-blind active treatment, each lasting eight weeks, and separated by a second single-blind wash-out placebo period of three weeks. The placebo periods were regarded as the baseline before the active treatment periods. During the active treatment periods the patients inhaled nedocromil sodium, 4 mg q.i.d. (Fisons Ltd., Loughborough, UK) or beclomethasone dipropionate, 200 μg q.i.d. (Glaxo Ltd., The Netherlands) from a metered dose inhaler. During the study patients were allowed to inhale salbutamol from a metered dose inhaler as rescue medication. No other anti-asthma drugs were allowed during the trial.

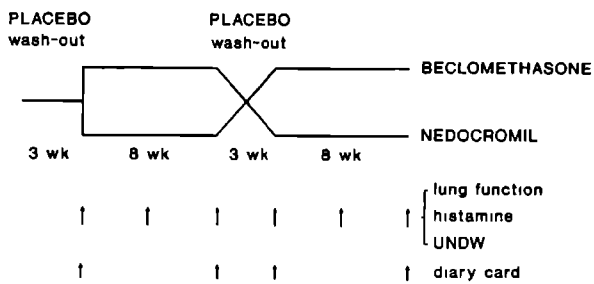

Figure 7.1: Study design of the double-blind, randomized crossover comparison of nedocromil sodium and beclomethasone in atopic asthmatic patients.

Measurements. At the end of both placebo periods and after 4 and 8 weeks of active treatment, a histamine provocation test and an ultrasonically nebulized distilled water (UNDW) provocation test were performed on two different days with at least one day in between to avoid histamine-induced tachyphylaxis for UNDW-induced bronchoconstriction [20]. The variation in the pre-challenge FEV_1 on these two days had to be within 10 %. Salbutamol was withheld for a period of at least 8 hours before each test and the trial medication was stopped for a period of at least 24 hours to avoid any direct drug effects on the provocation tests.

Lung Function. Flow-volume curves were performed to measure lung function (Pneumoscreen II, Jaeger, Würzburg, FRG) before and during each provocation test. The mean of the FEV_1 values before the UNDW and histamine provocation tests in each period was regarded as the pre-challenge FEV_1.

Histamine provocation tests were performed according to Ryan et al. [21]. The patients inhaled doubling doses of histamine (0.03 - 16 mg/ml) from a dosimeter (Jaeger, Würzburg, FRG). Six maximal inspirations were used to deliver 45 μl of histamine per dose. Inhalation of a concentration of 45 μl of 1 mg/ml resulted in a dose of 0.15 μmol. Flow-volume curves were recorded at 30, 90 and 180 seconds after inhalation. The PD_{20}histamine was calculated from pre-challenge values by linear interpolation on a semi-logarithmic curve.

UNDW provocation tests were performed with the Ultraneb 99 ultrasonic nebulizer (DeVilbiss, Sommerset, USA), according to a modified method described by Anderson et al. [6].

The output was fixed at 2 ml/min, which output was measured when the equipment was not attached. Air with UNDW was inhaled through a mouthpiece with tightened lips and nose clipped. A Leardal IV 2-way valve (Stavanger, Norway), with a dead space of 24 ml, was placed between the aerosol hose and the mouthpiece. A respirometer (British Oxygen Company, London, UK) was connected to the expiratory port of the two-way valve to measure the total volume of inhaled air. After inhalation of 20 liters of ambient air through the system, doubling volumes of air with UNDW (3, 5, 10, 20, 40, 80 and 160 liters) were inhaled at 5-minute intervals. Flow-volume curves were recorded 30, 90

and 180 seconds after inhalation. The test
was stopped when the last dose of air with UNDW, i.e. 160 l, was inhaled or a 20% fall in FEV_1 was achieved. Before and after each test the nebulizer chamber and aerosol hose were weighed and the total amount of inhaled UNDW was measured in ml H_2O. The PD_{20}UNDW, the cumulative dose of inhaled distilled water in ml H_2O causing a 20% fall in FEV_1 from post-air values, was calculated by linear interpolation on a semi-logarithmic curve.

Diary cards. Morning and evening peakflow measurements were recorded with a mini-Wright peakflow meter, the best of three attempts, and daily use of bronchodilators was registered as the total number of inhalations of salbutamol during the last 2 weeks of the placebo and the active treatment periods.

Statistical Analysis. PD_{20}histamine and PD_{20}UNDW data, FEV_1 values, and data obtained from diary cards were analyzed by the Wilcoxon signed rank test. For multiple comparisons a Bonferroni correction was used. The shift in PD_{20} values was calculated as the difference between the real baseline values and the values after 4 and 8 weeks of treatment. The changes in PD_{20} values were also expressed as doubling doses of inhaled histamine and UNDW, calculated from the individual baseline values. Period effects and carry-over effects were analyzed according to Pocock [22] by the Mann-Whitney U test. Correlations were calculated by the Spearman-rank test. FEV_1 values are presented as percentage of predicted [19]. Data are presented as mean $\pm$ standard error (SE) and statistical significance was accepted for $p < 0.05$.

7.4 RESULTS

The study was completed by 23 patients. Five patients (nrs.2,10,16,17 and 28) were unable to come to the laboratory for lung function and provocation tests at the appointed intervals and withdrew from the study voluntarily during the first active treatment period. Patient nrs. 2, 16, 17 and 28 had started with beclomethasone and nr. 10 had started with nedocromil sodium. None of the patients failed to complete the study due to an exacerbation of their asthma or the need of additional medication. One patient

Table 7.2: Carry-over and period effects of the two active treatment periods.

	group I(*)	group II(*)	carry-over effect	period effect
	Geometric mean	Geometric mean		
PD_{20}histamine (μmol)				
	(n=11)	(n=12)		
BDP				
baseline	0.02	0.07		
8 wks	0.30	0.44		
NS				
baseline	0.03	0.06		
8 wks	0.12	0.15	p=0.49	p=0.67
PD_{20}UNDW (ml)				
BDP				
baseline	0.9	1.7		
8 wks	4.1	6.4		
NS				
baseline	1.7	2.0		
8 wks	1.8	1.0	p=0.38	p=0.32
FEV_1 (% pred.)				
BDP				
baseline	79	81		
8 wks	91	89		
NS				
baseline	82	82		
8 wks	86	85	p=0.36	p=0.81
$ß_2$-agonist use (puffs/day)				
	(n=8)	(n=10)		
BDP				
baseline	2.3	5.5		
8 wks	2.0	2.5		
NS				
baseline	3.6	3.2		
8 wks	5.5	4.5	p=0.29	p=0.15

(*) Group I represents the patients who started with beclomethasone, group II represents the patients who started with nedocromil sodium. The data are expressed as geometric means.

Table 7.3: FEV_1 and PD_{20} values, peakflow rate in the morning and the evening, and β_2-agonist use during baseline and after 4 and 8 weeks of treatment.

	baseline	4 wk treatment	8 wk treatment
beclomethasone			
FEV_1 (% predicted)	79.8 ±3.7	89.6 ±3.4**	89.5 ±3.0**♦
PD_{20}histamine (µmol)	0.04 ±0.03	0.28 ±0.13***♦♦	0.37±0.18***♦♦♦
PD_{20}UNDW (ml)	1.3 ± 0.6	5.3 ±2.2***♦	6.2 ±2.5***♦♦
peakflow (l\min)			
(morning)	459 ±23		494 ±26*♦
(evening)	470 ±22		505 ±25*♦
β_2-agonist use (puffs/day)	3.3 ±0.8		2.2 ±0.5**♦♦
nedocromil sodium			
FEV_1 (% predicted)	82.4 ±3.7	86.4 ±3.0	84.8 ±3.3
PD_{20}histamine (µmol)	0.05 ±0.04	0.10 ±0.03**	0.13 ±0.07***
PD_{20}UNDW (ml)	1.9 ±0.7	2.7 ±1.5	1.8 ±2.0
peakflow (l/min)			
(morning)	468 ±24		471 ±23
(evening)	489 ±22		492 ±23
β_2-agonist use (puffs/day)	3.4 ±0.8		4.9 ±0.8

data are presented as geometric mean ± SE; * = $p<0.05$, ** = $p<0.005$ and *** = $p<0.0005$ versus the baseline; ♦ = $p<0.05$, ♦♦ = $p<0.005$ and ♦♦♦ = $p<0.0005$ versus nedocromil sodium.

(nr 23) did not react to UNDW. The diary cards of 18 patients could be evaluated for peakflow measurements and β_2-agonist use.

Of the 23 patients who completed the study, 11 patients started with beclomethasone and 12 with nedocromil sodium during the first active drug period. There were no period or carry-over effects for all parameters as shown in table 7.2.

None of the parameters, i.e FEV_1 values, PD_{20}histamine, PD_{20}UNDW, β_2-agonist use and peakflow measurements were significantly different in the two baseline periods (table 7.3).

Treatment with beclomethasone induced a significant improvement of the mean FEV_1 and decreased bronchial hyperresponsiveness to histamine and UNDW (table 7.3 and figure 7.2) after 4 and 8 weeks of treatment as compared to the baseline values.

Nedocromil sodium decreased bronchial hyperresponsiveness to histamine, but not to UNDW after 4 and 8 weeks of treatment and did not improve FEV_1 as compared to the baseline values during placebo treatment.

Treatment with beclomethasone was significantly better for all parameters, except for the baseline FEV_1 after 4 weeks of treatment.

The changes in PD_{20}histamine and PD_{20}UNDW, expressed as doubling doses, are presented in table 7.4. There was no significant difference in doubling doses between histamine and distilled water.

Table 7.4: Changes in PD_{20}histamine and PD_{20}UNDW calculated from baseline values and expressed as doubling doses.

	4 wk treatment	8 wk treatment
beclomethasone		
PD_{20}histamine	2.5 $\pm$ 0.4*	2.9 $\pm$ 0.4*
PD_{20}UNDW	1.8 $\pm$ 0.3**	2.3 $\pm$ 0.4**
nedocromil sodium		
PD_{20}histamine	1.2 $\pm$ 0.3	1.4 $\pm$ 0.3
PD_{20}UNDW	0.7 $\pm$ 0.2	0.8 $\pm$ 0.3

data are presented as mean $\pm$ SE, * = $p<0.05$, and ** = $p<0.01$ and versus nedocromil sodium

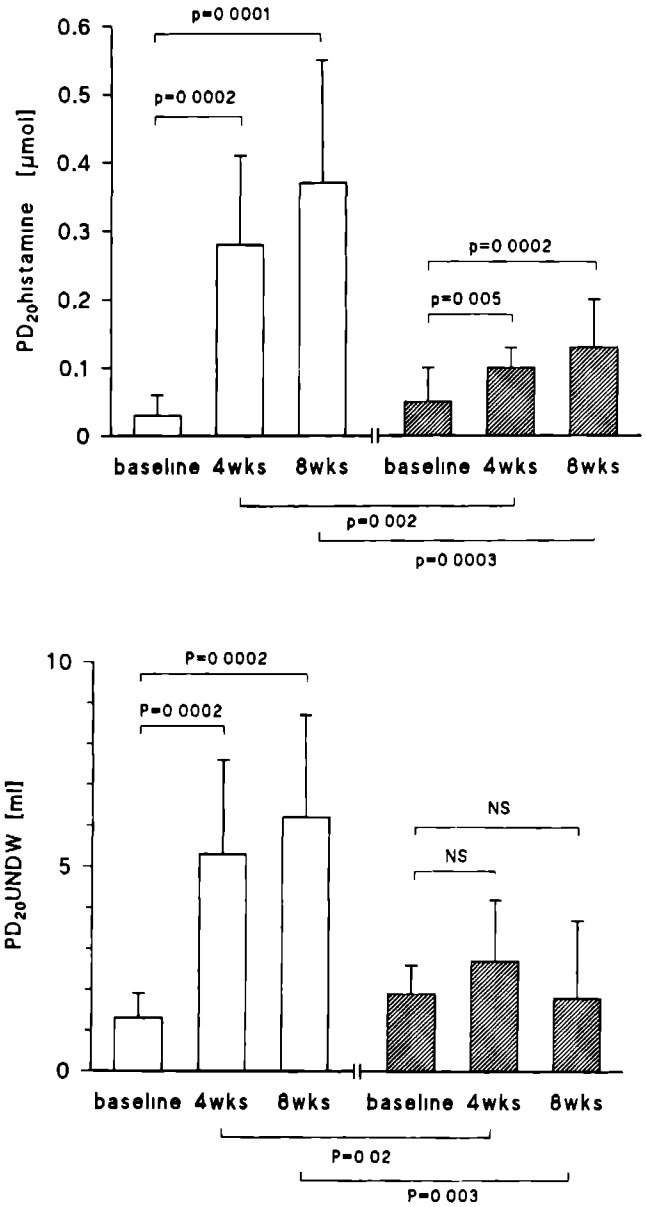

Figure 7.2: Geometric means (± SE) of the PD_{20}histamine (panel A) and PD_{20}UNDW (panel B) during baseline and after 4 and 8 weeks of treatment with beclomethasone (open bars) and nedocromil sodium (shaded bars).

A significant correlation was found between PD_{20}histamine and PD_{20}UNDW during placebo (r_s=0.76) and after 8 weeks of treatment with beclomethasone (r_s=0.73) and nedocromil sodium (r_s=0.87)($p<0.005$ for the three periods). The shift in PD_{20}histamine and PD_{20}UNDW after 4 weeks of treatment with beclomethasone showed no correlation, whereas after 8 weeks of treatment a significant correlation (r_s=0.64, p=0.004) was found. There were no correlations between the PD_{20} values for histamine and UNDW and the FEV_1.

Both morning and evening peakflow rates were significantly increased and $ß_2$-agonist use was significantly decreased after 8 weeks of treatment with beclomethasone compared to the baseline values and treatment with nedocromil sodium (table 7.3 and figure 7.3)

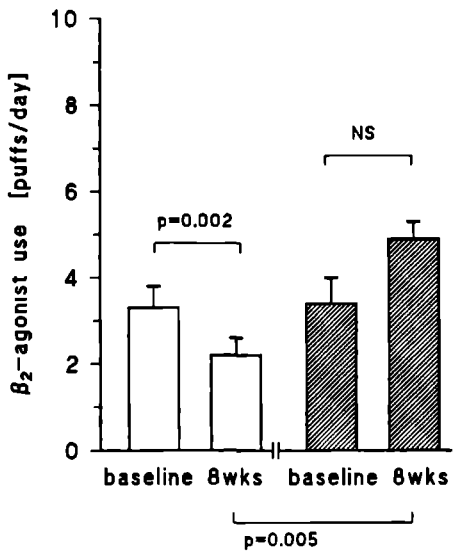

Figure 7.3: Geometric means (± SE) of the $ß_2$-agonist use during the last week of the baseline period and beclomethasone (open bars) and nedocromil sodium treatment (shaded bars).

7.5 DISCUSSION

The results of our study demonstrate that, in patients with allergic asthma, treatment with inhaled beclomethasone in a total daily dose of 800 μg improves lung function and decreases bronchial hyperresponsiveness to histamine and distilled water provocation, after 4 and 8 weeks of treatment. Beclomethasone also reduces the need for additional bronchodilators as reflected by a decrease in daily use of salbutamol. Nedocromil sodium with a total daily dose of 16 mg decreases bronchial hyperresponsiveness to histamine, but not to UNDW, after 4 and 8 weeks of treatment. Nedocromil sodium had no effect on lung function and $ß_2$-agonist use either. Bronchial hyperresponsiveness and most lung function parameters were significantly better during treatment with beclomethasone than with nedocromil sodium.

Our results are partly comparable with those of a study in non-allergic patients with mild asthma [23], comparing a total daily dose of 400 μg beclomethasone with 16 mg nedocromil sodium. The authors measured a significant decrease in bronchial hyperresponsiveness to methacholine after 4 and 8 weeks of treatment with beclomethasone and after 8 weeks of treatment with nedocromil sodium. Only beclomethasone could induce a significant increase in lung function, but no significant diffences were found between beclomethasone and nedocromil sodium [23].

In the present study beclomethasone induced a significant increase in both morning and evening peakflow rates, whereas nedocromil sodium had no significant effects. These findings are in contrast with those of a study comparing beclomethasone 400 μg daily with nedocromil sodium 16 mg daily in a group of 13 asthmatic subjects [24]. This study showed that after 8 weeks of treatment with both beclomethasone and nedocromil sodium there were significant increases in morning and evening peakflow rates. $ß_2$-agonist use was lower during beclomethasone treatment, whereas nedocromil sodium had no significant effect.

The significantly better effect of beclomethasone, compared to nedocromil sodium, on bronchial hyperresponsiveness, lung function, and $ß_2$-agonist use in our group of atopic asthmatic patients is probably related to the dose of beclomethasone used and the

characteristics of our patients with respect to their degree of airway hyperresponsiveness. Other studies [23-25], who investigated a lower dose of beclomethasone, i.e. 400 μg daily, and an equal amount of nedocromil sodium, i.e. 16 mg, could not demonstrate significant differences between both treatments. A further difference between the these studies [23-25] and ours is the degree of bronchial hyperresponsiveness. Twenty-three of our patients used inhaled corticosteroids before entering the study, in contrast to a minority of the patients from the studies referred to [23-25]. This may indicate more severe asthma in our group of patients. Nevertheless, nedocromil sodium in a total daily dose of 16 mg seems to be less potent in this group of patients. Comparing the duration of treatment needed to achieve significant effects on asthma, beclomethasone-induced changes can be demonstrated after 3 to 4 weeks of treatment, whereas the effects of nedocromil treatment become clear after 4 to 8 weeks of treatment, as can be concluded from our data and the data from other studies [23-25]. During beclomethasone treatment the bronchial hyperresponsiveness to histamine further improved between 4 and 8 weeks of treatment, whereas this effect could not be demonstrated for nedocromil sodium.

Beclomethasone treatment significantly improved both FEV_1 and bronchial hyperresponsiveness to histamine and UNDW. This may suggest that the improvement in bronchial hyperresponsiveness is partly the result of increase in lung function. Although a correlation between the degree of airway obstruction and bronchial hyperresponsivenss has been found in a heterogeneous population [26], this correlation does not seem to exist in asthmatic subjects in contrast to patients with a chronic airway obstruction [27]. As for asthmatics, this is confirmed by our data, since we also could not demonstrate a correlation between pre-challenge FEV_1 and the PD_{20}histamine or PD_{20}UNDW. Hence, in asthma the pre-challenge FEV_1 seems to be a relatively minor determinant for the improvement of bronchial hyperresponsiveness.

PD_{20}histamine and PD_{20}UNDW showed a good correlation during the trial. The shifts in PD_{20} values for histamine and UNDW were not significantly different. Therefore, it appears that histamine and UNDW are equally sensitive in detecting changes in bronchial hyperresponsiveness induced by anti-inflammatory drugs as beclomethasone in asthmatic patients. However, during beclomethasone treatment there was no correlation between the change in PD_{20}histamine and PD_{20}UNDW after 4 weeks of treatment, whereas

after 8 weeks of treatment a significant correlation was found. Furthermore, the increase in PD_{20}histamine was significantly more pronounced after 8 weeks than after 4 weeks of treatment with beclomethasone. This indicates that beclomethasone-induced effects in asthma are probably measured by histamine and UNDW challenge on a different level of bronchial responsiveness. These findings are supported by what we know about the underlying mechanisms in histamine- and UNDW-induced bronchoconstriction, which are not identical. The histamine bronchoconstrictor response is mainly a direct effect of the drug on the airway smooth muscles [7], whereas in UNDW-induced bronchoconstriction also the release of mediators from inflammatory cells, like mast cells, seems to be involved [28]. Preinhalation of sodium cromoglycate can totally block UNDW-induced bronchoconstiction and prevent mediator release [6,29], but it has no effect on histamine-induced bronchoconstriction [30].

Nedocromil sodium also has an inhibitory effect on UNDW-induced bronchoconstriction when inhaled 30 minutes before the challenge [14,31]. The duration of this protective effect in UNDW provocation is unknown. In exercise testing the protective effect of nedocromil sodium lasts at least 2 hours [17]. We arbitrarily stopped beclomethasone and nedocromil sodium for a period of 24 hours before histamine and UNDW challenge to prevent a direct blocking effect on the bronchoprovocation tests. The lack of a significant change in PD_{20}UNDW during nedocromil sodium indicates that after 24 hours the direct inhibitory effect, probably caused by a blockade of mediator release, has disappeared. The beneficial effects of beclomethasone was not influenced by the 24 hour withdrawal period. In contrast to the inhaled corticosteroids, nedocromil sodium in this dose appears to have no long-lasting effects on mediator release, as measured by UNDW-induced bronchoconstriction. The sole effect of nedocromil sodium treatment in our study was a decrease in bronchial hyperresponsivenss to histamine. The mode of action of long-term effects of nedocromil sodium in asthmatic subjects is not known. This decrease in bronchial hyperresponsiveness to histamine probably indicate a reduction of the inflammation in the airway smooth muscles, although treatment with nedocromil sodium could not improve the other parameters.

Topically administered corticosteriods have shown to reduce the number of mast cells in the skin and diminish the measurable histamine release dramatically [32]. In asthmatics,

corticosteroid treatment induces a significant fall in whole blood histamine, a mast cell mediator [33]. Thus, corticosteroids appear to deplete or reduce the stores of histamine in tissue [34,35] and probably modify mediator release [35]. This may partly explain the difference of treatment effects of both drugs on UNDW-induced bronchoconstriction.

We conclude that nedocromil sodium, 16 mg daily, has anti-asthmatic properties in this group of allergic asthmatic patients, as demonstrated by a significant decrease in bronchial hyperresponsiveness to histamine after 4 and 8 weeks of treatment. Beclomethasone, 800 μg daily, however, showed superior effects compared to nedocromil sodium with regard to the decrease in β_2-agonist use after 8 weeks of treatment, the increase in lung function after 4 and 8 weeks and the decrease of bronchial hyperresponsiveness measured by histamine and UNDW after 4 and 8 weeks of treatment.

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CORRELATIONS OF SYMPTOM SCORES AND β_2-AGONIST USE WITH BRONCHIAL HYPERRESPONSIVENESS TO HISTAMINE AND DISTILLED WATER AND PERCEPTION OF DYSPNOEA DURING HISTAMINE AND DISTILLED WATER CHALLENGE.

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submitted for publication

8.1 ABSTRACT

We studied the correlations between asthma symptom scores, β_2-agonist use and bronchial hyperresponsiveness to histamine and distilled water, during a double-blind, crossover study comparing 8 weeks of treatment with beclomethasone to that of nedocromil sodium. Before each active treatment the patients used placebo, as wash-out, for a period of 3 weeks. Asthma symptom scores were evaluated in 18 allergic asthmatic subjects. Eight weeks of treatment with beclomethasone significantly decreased daytime dyspnoea ($p=0.002$) and β_2-agonist use ($p=0.002$) as compared to nedocromil sodium. Furthermore, asthma symptom scores were correlated to the bronchoconstrictor responses induced by inhalation of histamine and UNDW. The distilled water-induced bronchoconstrictor response correlated slightly better or more consistently to daytime dyspnoea and β_2-agonist use than to histamine-induced bronchoconstriction. Assessment of the change in dyspnoea during bronchial challenge demonstrated a significant correlation between the increase in dyspnoea and the fall in FEV_1 during distilled water inhalation, but not during inhalation of histamine.

These results indicate that differences in pathophysiologic mechanisms underlying bronchial hyperresponsiveness to histamine and distilled water are reflected in their relationship to β_2-agonist use and asthma symptoms score registered on diary cards and during bronchial challenge. Distilled water-induced bronchoconstriction correlates slightly better and more consistently to asthma symptom scores and β_2-agonist use than histamine.

8.2 INTRODUCTION

Asthma symptom scores and bronchial hyperresponsiveness to non-specific stimuli indicate asthma severity [1]. However, assessment of asthma severity by recording symptom scores and questionnaires may not be a sensitive tool for discrimination between subjects with and without asthma or bronchial hyperresponsiveness to histamine and methacholine in epidemiologic surveys [2-4]. In asthmatic patients Brooks et al [5] found a

significant correlation between severity of complaints deduced from a specialised questionnaire and bronchial hyperresponsiveness to methacholine. In a previous study Molema et al.[6] investigated the correlations between asthma symptoms and bronchial hyperrresponsiveness to histamine and exercise during placebo and budesonide treatment. Only a consistent correlation between β_2-agonist use and PC_{20}histamine could be demonstrated, whereas correlations to asthma symptoms were poor. Physical stimuli like inhaled distilled water have been suggested to correlate better with asthma symptoms because of their similarity to naturally occurring non-specific stimuli [7]. As discussed in chapters 1 and 3 the pathophysiologic mechanisms underlying bronchial hyperresponsiveness to inhaled histamine and distilled water are different. Histamine and methacholine inhalation mainly induce bronchoconstriction through direct airway smooth muscle contraction and therefore assess reactivity and contractility of airway smooth muscle function [7]. In UNDW-induced bronchoconstriction the release of inflammatory mediators and vagal reflexes are also involved. Thus, distilled water inhalation assesses a more complete pathway of bronchial hyperresponsiveness [7]. The aim of this study was to investigate whether differences in underlying mechanisms of bronchial hyperresponsiveness to histamine and distilled water are reflected in differences in their relationship with asthma severity as measured by symptom scores, i.e. daytime dyspnoea, nighttime dyspnoea and β_2-agonist use. These tests were performed during long-term treatment with beclomethasone and nedocromil sodium in allergic asthmatic subjects. Furthermore, asthma symptoms were recorded during bronchial challenge with histamine and distilled water to investigate symptom perception during increasing bronchoconstriction.

8.3 PATIENTS AND METHODS

Subjects. Twenty-eight non-smoking asthmatic subjects [8], with a reversibility $\geq$ 15% in response to an inhaled β_2-agonist, participated in the study which was a part of the study described in chapter 6. All patients were atopic, defined as two or more positive intracutanous skin test reactions to common airborne allergens. The study was approved

by the local Ethics Committee and all patients gave their written informed consent.

Study Design. In a randomized crossover, double-blind, double-dummy study, 8 weeks of treatment with nedocromil sodium, 4 mg q.i.d. (Fisons Ltd., Loughborough, UK) was compared to 8 weeks of treatment with beclomethasone diproprionate, 200 μg q.i.d. (Glaxo Ltd, The Netherlands), both inhaled from a metered dose inhaler.
A single-blind three-week placebo wash-out period was followed by two periods of active treatment, each lasting eight weeks, and which was separated by a second placebo wash-out period of three weeks, as shown in figure 6.1. The placebo wash-out periods were concidered as baseline periods. Salbutamol was allowed as rescue medication. No other anti-asthma drugs were used during the study.

At the end of both placebo periods and after 4 and 8 weeks of active treatment, a histamine provocation test [9] and an UNDW provocation test [10] were performed to assess bronchial hyperresponsiveness. Salbutamol was withheld for at least 8 hours before each test and the trial medication was stopped for at least 24 hours.

To investigate the perception of dyspnoea during histamine and distilled water inhalation the patients recorded the severity of dyspnoea and coughing on a visual analog scale (VAS) [11], with a length of 10 cm, five minutes before the bronchoprovocation tests and at the deepest fall in FEV_1 during the challenge. The increase in dyspnoea and coughing during the tests was calculated in cm from baseline values. The fall in FEV_1 during the tests was calculated in percentage fall from baseline values.

Diary card. The patients recorded daytime and nighttime dyspnoea, coughing and β_2-agonist use on a diary card during the last two weeks of the placebo and active treatment periods. The symptom scores during the last week of each period were used for statistical analysis. The severity of dyspnoea symptoms during the day and during the night were registered on a visual analog scale (VAS) [11]. The daily use of bronchodilators was recorded by the patients as the total number of inhalations of salbutamol.

Statistical Analysis. The diary card data and treatment effects were analyzed by the Wilcoxon test with Bonferroni correction. Correlations were calculated by the Spearman-rank test. Comparison of correlations were performed by comparing the involved cross-products by the Wilcoxon test. Baseline FEV_1 values were presented as percentage of predicted [12]. All data are presented as mean $\pm$ SE and statistical

significance is accepted for $p < 0.05$.

Table 8.1: Spearman correlation coefficients between daytime dyspnoea (D_D), nighttime dyspnoea (N_D), β_2-agonist use (β_2), PD_{20}histamine ($PD_{20}H$) and the PD_{20}UNDW ($PD_{20}U$).

BASELINE PERIOD 1			
$PD_{20}U$	0.76**		
D_D	-0.36		-0.47
N_D	-0.28		-0.39
β_2	-0.45		-0.55*
	$PD_{20}H$		$PD_{20}U$
BECLOMETHASONE			
$PD_{20}U$	0.71**		
D_D	-0.44		-0.57*
N_D	-0.09		-0.11
β_2	-0.42		-0.61*
	$PD_{20}H$		$PD_{20}U$
BASELINE PERIOD 2			
$PD_{20}U$	0.75**		
D_D	-0.66*		-0.75**
N_D	-0.54*		-0.51*
β_2	-0.68*		-0.77**♦
	$PD_{20}H$		$PD_{20}U$
NEDOCROMIL SODIUM			
$PD_{20}U$	0.94**		
D_D	-0.77**	-0.70*	
N_D	-0.16		-0.21
β_2	-0.60*		-0.53*
	$PD_{20}H$		$PD_{20}U$

* $p<0.05$; ** $p<0.005$;
♦ $p<0.05$ vs $PD_{20}H$.

8.4 RESULTS

Twenty-three patients completed the study as described in paragraph 7.4. The diary cards of 17 patients could be evaluated for asthma symptoms.

Diary card. No differences were found between daytime dyspnoea, nighttime dyspnoea, coughing and β_2-agonist use recorded on the diary cards during both baseline periods. The treatment effects on β_2-agonist use have already been described in chapter 7. Coughing did not change during placebo or active treatment. Daytime and nighttime dyspnoea, and β_2-agonist use were significantly lower after eight weeks of treatment with beclomethasone than during the baseline period (p=0.01, p=0.002 and p=0.002, respectively) (figures 8.1 and 7.3).

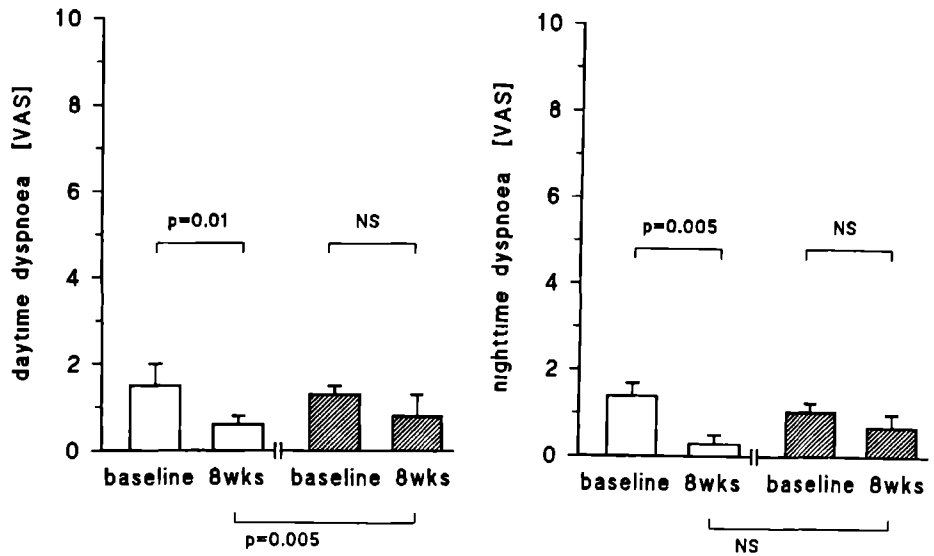

Figure 8.1: Mean values (± SE) for daytime (panel A) and nighttime dyspnoea (panel B), registered on a visual analog scale during the last week of baseline and beclomethasone (open bars) and nedocromil sodium treatment (shaded bars).
NS: not significant.

Nedocromil sodium, however, could not improve these parameters after 8 weeks of treatment compared to the baseline. After 8 weeks of treatment daytime dyspnoea and β_2-agonist use were significantly lower during beclomethasone than during nedocromil sodium.

The correlations between the asthma symptom scores derived from the diary cards and PD_{20}histamine and PD_{20}UNDW are shown in table 8.1. Throughout the whole study PD_{20}histamine and PD_{20}UNDW were significantly correlated. In all four periods β_2-agonist use was also consistently correlated to the PD_{20}UNDW (table 8.1), but to the PD_{20}histamine. During beclomethasone treatment the PD_{20}UNDW was significantly correlated to daytime dyspnoea and β_2-agonist use, whereas there was no correlation with the PD_{20}histamine for these parameters. In baseline period 2, correlations were found between all parameters and the PD_{20} values. The correlation coefficient between PD_{20}UNDW and β_2-agonist use was significantly higher than that between PD_{20}histamine and β_2-agonist use ($p=0.03$). During nedocromil sodium treatment both PD_{20}histamine and PD_{20}UNDW showed a significant correlation to daytime dyspnoea and β_2-agonist use. No correlations were found between coughing and PD_{20} values. Significant correlations were found between coughing and nighttime dyspnoea in all periods ($r_s=0.69$, $r_s=0.49$, $r_s=0.65$ and $r_s=0.63$ respectively, $p<0.05$). Daytime dyspnoea and coughing showed only a significant correlation during both placebo periods ($r_s=0.64$ and $r_s=0.65$ respectively, $p<0.05$), but not during active treatment.

Daytime dyspnoea and β_2-agonist use showed significant correlations in all four periods ($r_s=0.87$, $r_s=0.73$, $r_s=0.84$ and $r_s=0.49$ respectively, $p<0.05$).

Perception of dyspnoea during bronchial challenge. The dyspnoea scores during the bronchoprovocation tests could be evaluated in 19 patients. There was no difference in increase in dyspnoea and fall in FEV_1 during histamine and distilled water challenge (figure 8.2). However, during distilled water challenge the increase in coughing was significantly more pronounced than during histamine inhalation ($p=0.002$). There was no significant correlation between the PD_{20} values and the increase in dyspnoea and coughing during the tests.

The correlations between the increase in dyspnoea and the fall in FEV_1 during histamine and UNDW challenge are shown in figure 8.3 for baseline and active treatment periods.

During distilled water inhalation there was a significant correlation between the increase in dyspnoea and fall in FEV_1 in all four periods, whereas during histamine challenge no correlations were present.

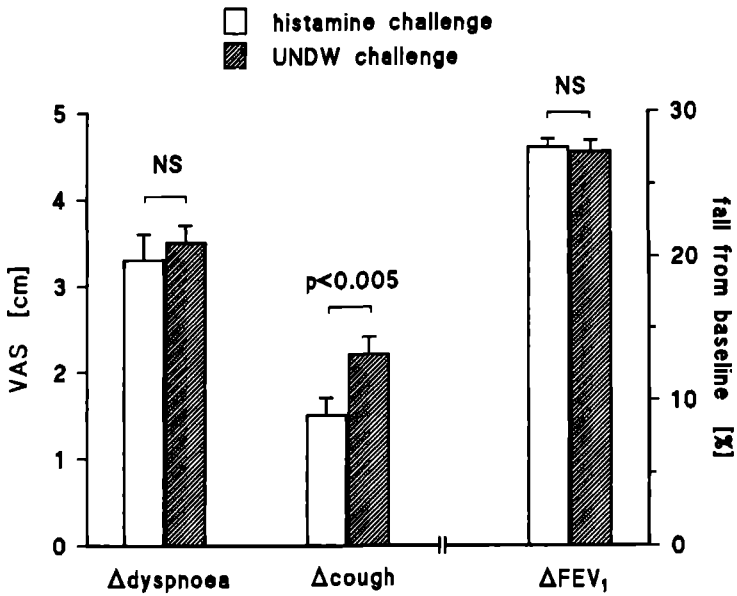

Figure 8.2: Mean increases (± SE) in dyspnoea and cough, registered on a visual analog scale, and mean falls in FEV_1 during histamine and distilled water challenge in allergic asthmatic subjects. NS: not significant.

No correlation could be found between the increase in coughing and the fall in FEV_1 during either of the tests.

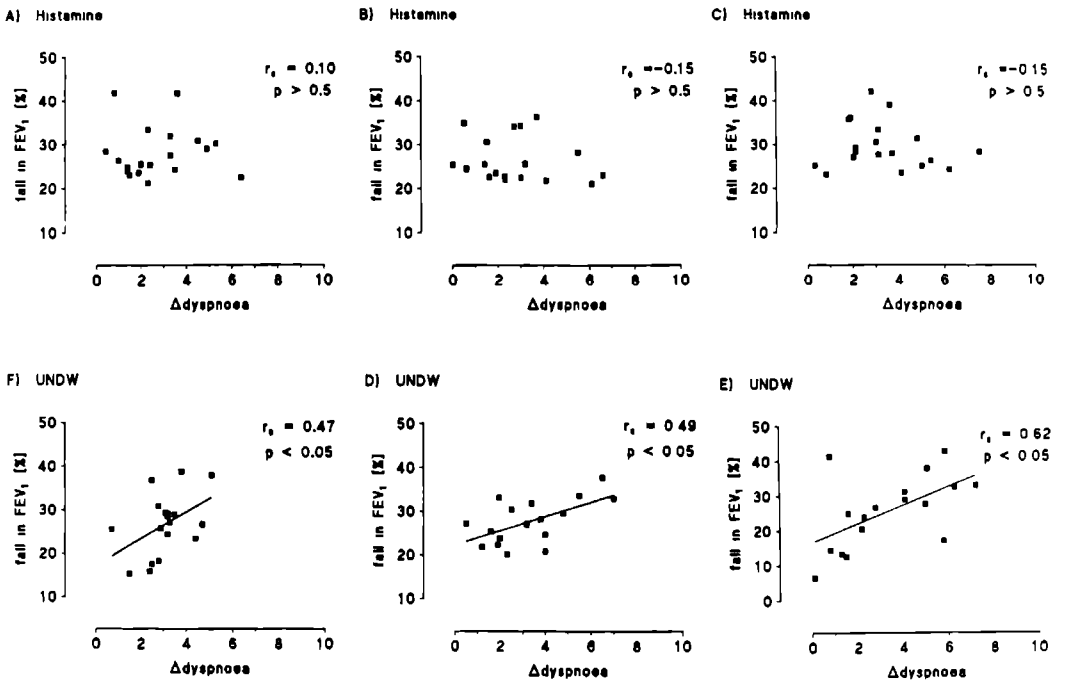

Figure 8.3: The relationship between the fall in FEV_1 and increase in dyspnoea caused by histamine and distilled water challenge during baseline (A and D), beclomethasone (B and E) and nedocromil sodium treatment (C and F)

8.5 DISCUSSION

Our data, presented in this chapter, demonstrated a significant decrease in daytime and nighttime dyspnoea and β_2-agonist use during beclomethasone compared to baseline values, whereas nedocromil sodium had no effect on these parameters.

Although there was a good correlation between the histamine- and UNDW-induced bronchoconstrictor response throughout the whole study, distinct differences were found between the correlations of the two bronchoconstrictor responses and the asthma symptom scores as recorded on the diary cards. In baseline period 1, the PD_{20}UNDW significantly correlated to β_2-agonist use and during beclomethasone treatment the PD_{20}UNDW significantly correlated to daytime dyspnoea and β_2-agonist use, whereas PD_{20}histamine did not correlate in these cases. In baseline period 2, the correlation between β_2-agonist use and PD_{20}UNDW was significantly stronger than that between β_2-agonist and PD_{20}histamine. The fact that asthma symptom scores correlates slightly better with PD_{20}UNDW than with PD_{20}histamine, supports the idea that bronchoprovocation with the physical stimulus UNDW, correlates better with asthma symptoms than pharmocological stimuli like histamine do [7,13].

During the study there was a consistent relationship between β_2-agonist use and daytime dyspnoea, indicating that the patients were using their bronchodilator medication on demand. Furthermore, this correlation indicates that additional β_2-agonist use reflects the severity of dyspnoea and indirectly the severity of asthma, as pointed out by others [1,6]. Also β_2-agonist use and PD_{20}UNDW showed a consistent correlation, which was not affected by treatment-induced changes of the parameters involved.

During bronchial challenge with distilled water the increase in dyspnoea significantly correlated to the fall in FEV_1 in all treatment periods, whereas during histamine challenge no correlations were found between both parameters. Apparently the perception of dyspnoea during histamine challenge in this group of patients is not related to the decrease in airflow. As mentioned above, the pathophysiologic mechanisms underlying the histamine- and distilled water-induced bronchoconstrictor response are

clearly different. Involvement of mediator release or vagally mediated reflexes [14,15] may well contribute to a better perception of changes in lung function during distilled water-induced bronchoconstriction than during histamine.

In this study the magnitude of the increase in dyspnoea and fall in FEV_1 were not significantly different in the two tests. The increase in coughing during distilled water inhalation was significantly more pronounced than during histamine inhalation. Both cough and bronchoconstrictor reflexes are closely related and can potentiate each other [16]. Cough receptors can be stimulated independently from bronchconstriction, but they may also be caused by a bronchoconstrictor response [16]. Inhalation of irritant aerosols induces coughing in both normal and asthmatic subjects, which implies that coughing is a normal phenomenon [17]. Thus, coughing, although registered by the patients considerably more during distilled water inhalation, seems not to be related to a better perception of dyspnoea.

We conclude that asthma symptom scores recorded on diary cards correlate slightly better with UNDW- than with histamine-induced bronchoconstriction. A consistent correlation was found between bronchial hyperesponsiveness to distilled water and $ß_2$-agonist use. During bronchial challenge with distilled water the change in dyspnoea significantly correlated with the fall in FEV_1, but not during histamine challenge. These results suggest a closer relationship between asthma symptoms and bronchial hyperresponsiveness to UNDW than between asthma symptoms and bronchial hyperresponsiveness to histamine.

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SUMMARY AND CONCLUSIONS

In ***Chapter 1*** a general introduction to this thesis is given and an outline of the aims of the different studies.

Asthma is a disease characterized by paroxysms of dyspnoea, wheezing and cough caused by an increased bronchial hyperresponsiveness. This hyperresponsiveness is defined as an exaggerated bronchoconstrictor response of the airways on exposure to a small quantity of non-specific stimuli which do not provoke such a reaction in normal subjects. Bronchial hyperresponsiveness is related to inflammation in the airways, diurnal variation in lung function and reversibility of bronchoconstriction to β_2-agonsts. Asthma severity and the clinical prognosis of asthma with regard to the development of chronic obstructive pulmonary disease and further decline in lung function are related to the severity of bronchial hyperresponsiveness. The assessment and pharmacological modulation of bronchial hyperresponsiveness in asthmatic subjects is the main subject in this thesis.
Bronchial hyperresponsiveness is usually assessed by provocation with histamine or methacholine inhalation challenges. Histamine and methacholine mainly act directly on airway smooth muscles, in contrast to physical stimuli which act indirectly. In susceptible subjects provocation with physical stimuli causes mediator release in the airway wall, finally resulting in airway smooth muscle contraction. Thus, physical stimuli may assess a more complete pathway of bronchial hyperresponsiveness than pharmacological stimuli. It has been suggested that these physical stimuli cause better correlations between bronchial hyperresponsiveness to UNDW and asthma symptoms than between bronchial hyperresponsiveness to histamine or methacholine and asthma symptoms.
The main purpose of this thesis is to investigate whether a physical stimulus like distilled water has advantages over histamine in the assessment of bronchial hyperresponsiveness in asthmatic subjects.

In ***Chapter 2*** we investigated the reproducibility of the histamine provocation test by means of the Astma Provocation System (APS), a breath-actuated dosimeter, in nine asthmatic patients. The histamine provocation test with the dosimeter, performed on two different days, showed a good reproducibility with a standard deviation for repeated measurements of 10.4%.
The APS dosimeter technique was further compared to the 2-minute tidal breathing tech-

nique with the Wright nebulizer in fifteen patients with dyspnoea and suspected bronchial hyperresponsiveness. The tidal breathing technique is a simple, and inexpensive method. However, various factors like flow rate of the nebulizer, breathing frequency, inspiration volume and breath-holding time must be standardized to get reproducible results. The dosimeter technique is a more sophisticated and more expensive method with the advantage that the exact provocative dose of inhaled agent is known.
Both techniques showed a good correlation ($r=0.93$, $p<0.0005$) and equally separated hyperresponsive from non-hyperresponsive subjects. The limit of agreement (-102% and + 83%) and 95% confidence limit ($\pm$27%) between the two techniques show that both techniques are not fully exchangeable.

In ***Chapter 3*** we reviewed the literature on distilled water-induced bronchoconstriction.

The mechanism underlying the distilled water-induced bronchoconstrictor response is probably multifactorial. The non-isotonicity of the aerosol appears to be the trigger for bronchoconstriction in asthmatics, whereas normal subjects do not show this reaction to hypotonic or hypertonic aerosols. The acidity and titrability of the acid enhance the distilled water-induced bronchoconstrictor response. The protective effect of atropine and a high dose of ipratropium bromide on the UNDW-induced bronchoconstrictor response suggests at least a partial involvement of the autonomic nervous system. Also the release of mediators from inflammatory cells, especially the mast cell, are involved. Cromoglycate and nedocromil sodium can totally inhibit the UNDW-induced bronchoconstriction. Furthermore, leukotrienes D_4 and E_4 may also play a role, since specific antagonists can diminish the UNDW-induced bronchoconstriction.

The UNDW-induced bronchoconstrictor response showes a significant correlation to histamine- and methacholine-induced bronchoconstriction in adults, but not in children. The effects of exercise and cold air hyperventilation are significantly correlated to UNDW-induced responses. Exercise-induced bronchoconstriction showed the closest relationship to the UNDW-induced bronchoconstrictor response, and a common pathophysiologic mechanism has been suggested.

In ***Chapter 4*** we described the protocol of the distilled water provocation test used in the present studies in detail and investigated their reproducibility, relation to histamine challenge and duration of bronchoconstrictor response after UNDW inhalation.

The reproducibility of the UNDW-induced bronchoconstrictor response was investigated with an short-term interval of 2 to 3 weeks and a long-term interval of approximately 3 months in two groups of 17 and 16 asthmatic patients respectively. To quantify the UNDW bronchoconstrictor response, several parameters derived from a semi-logarithmic dose-response curve were compared, i.e. the PD_{20}UNDW, the area under the curve (AUC), the reactivity and the sensitivity. The results showed a good short-term and long-term reproducibility of the UNDW-induced bronchoconstrictor response as measured with the PD_{20}UNDW, the sensitivity and AUC, whereas the reactivity was not reproducible. The PD_{20}UNDW and the sensitivity showed the least variation as measured with the Standard deviation for repeated measurements.

The duration of the UNDW-induced bronchoconstriction was investigated in 12 asthmatic patients. All patients showed a fall in FEV_1 of more than 20 % after UNDW inhalation. Although there was a wide range in spontaneous recovery after the maximal bronchoconstriction was obtained, the bronchoconstrictor effect decreased in all subjects within 5 minutes after inhalation of the distilled water.

In 16 atopic asthmatic subjects the distilled water-induced bronchoconstrictor response was compared to histamine induced-bronchoconstriction. The PD_{20}UNDW and PD_{20}histamine were significantly correlated ($r=0.72$, $p=0.002$).

In ***Chapter 5*** we investigated whether histamine inhalation could cause refractoriness to bronchoconstriction induced by UNDW or a second histamine challenge in nine asthmatic patients. Refractoriness or tachyphylaxis in bronchial challenge is a phenomenon of a substantially attenuated bronchoconstrictor reponse after re-exposure to the same stimulus within several hours after the first challenge. Pre-exposure to other stimuli can also cause refractoriness. In our study, preinhalation of histamine induced a significant diminished bronchoconstrictor response to UNDW. The mean PD_{20}UNDW increased from 3.5 ± 0.8 ml to 11.8 ± 2.6 ml after histamine challenge ($p < 0.01$). Repeated inhalation of histamine did not change the bronchoconstrictor response to his-

tamine within one hour after rechallenge. The magnitude of refractoriness to UNDW inhalation after preinhalation of histamine was correlated to the hyperresponsiveness to histamine ($r = 0.73$, $p < 0.05$). This indicates that histamine-induced refractoriness to UNDW seems to be related to the degree of bronchial hyperresponsiveness.

In ***chapter 6*** we investigated the protective properties of terbutaline and a high dose of ipratropium bromide on the UNDW-induced bronchoconstrictor response. UNDW-induced bronchoconstriction can be inhibited by $ß_2$-agonists, like fenoterol and salbutamol, drugs with mast cell stabilizing properties like cromoglycate, and anti-cholinergic drugs like atropine. Ipratropium bromide, however, in a clinical effective dose of 40 to 80 μg has been reported to have no effect on UNDW-induced bronchoconstriction. The protective properties of ipratropium bromide in UNDW challenge, therefore, are not clear. In a randomized, double-blind, placebo-controlled study, we investigated the protective effects of ipratropium bromide 160 μg and 320 μg and terbutaline 500 μg on UNDW-induced bronchoconstriction in nine stable asthmatic patients. To compare the drug effects we determined the PD_{20}UNDW and the reactivity and the sensitivity of the UNDW dose-response curve. Both drugs showed a significant increase ($p<0.001$) in baseline FEV_1 with no significant difference between the drugs or the doses of ipratropium bromide. Preinhalation of ipratropium bromide 320 μg and terbutaline 500 μg inhibited UNDW-induced bronchoconstriction as measured by PD_{20}-UNDW and sensitivity ($p<0.01$), whereas ipratropium bromide 160 μg had no protective effect. There was no correlation between the increase in baseline FEV_1 and the increase in PD_{20}UNDW, indicating that the protective effect on UNDW-induced bronchoconstriction is not dependent on the bronchodilation induced by terbutaline and ipratropium bromide. It is also concluded that the UNDW-induced bronchoconstriction is at least partly vagally mediated.

In ***Chapter 7*** the pharmacological modulation of bronchial hyperresponsiveness in atopic asthmatic subjects is investigated. In a randomized crossover, double-blind study we compared the effects of inhaled nedocromil sodium, 4 mg q.i.d., with inhaled be-

clomethasone dipropionate, 200 μg q.i.d. in 23 atopic asthmatic patients. After a 3-week single-blind wash-out placebo period and after 4 and 8 weeks of active treatment, drug effects were assessed with histamine and UNDW inhalation challenges, baseline lung function and $ß_2$-agonist use registered on a diary card. Treatment with beclomethasone caused a significant increase in baseline lung function ($p<0.005$) and daily peakflow measurements ($p<0.05$) and a decrease in bronchial hyperresponsiveness to histamine ($p<0.0005$) and UNDW ($p<0.0005$) as compared to the baseline. Nedocromil sodium decreased the histamine responsiveness ($p<0.005$), but not the UNDW responsiveness, and it did not improve baseline lung function and peak flow measurements. $ß_2$-agonist use was significantly diminished after an 8-week treatment with beclomethasone, whereas nedocromil sodium had no such an effect. The improvement in bronchial hyperresponsiveness to histamine and UNDW, the peakflow measurements and the changes in $ß_2$-agonist use were more pronounced for beclomethasone than for nedocromil sodium ($p<0.05$).

After 8 weeks of treatment with beclomethasone bronchial hyperresponsiveness to histamine had improved significantly more than after 4 weeks of treatment, whereas the PD_{20}UNDW was not significantly different after 4 and 8 weeks of treatment. The change in PD_{20}histamine correlated to the change in PD_{20}UNDW after 8 weeks of treatment but not after 4 weeks of treatment. These differences in time course of drug-induced changes indicate that histamine and distilled water challenge assess bronchial hyperresponsiveness on a different level, and they may confirm differences in the underlying mechanisms.

Chapter 8 describes the correlations between asthma symptom scores and $ß_2$-agonist use, both registered on a diary card, and bronchial hyperresponsiveness to histamine and distilled water, during the study described in chapter 7. Furthermore, asthma symptoms were recorded during bronchial challenge and correlated to changes in lung function during testing.

After 8 weeks of treatment with beclomethasone, asthma symptom scores registered on a diary card demonstrated a more significant decrease in daytime dyspnoea ($p=0.002$) and $ß_2$-agonist use ($p=0.002$) than after treatment with nedocromil sodium. The distilled water-induced bronchoconstrictor response correlated slightly better and

more consistently to daytime dyspnoea and β_2-agonist use than histamine-induced bronchoconstriction.

Assessment of the change in dyspnoea during bronchial challenge demonstrated a significant correlation between the increase in dyspnoea and the fall in FEV_1 during distilled water inhalation but not during inhalation of histamine.

These results indicate that differences in pathophysiologic mechanisms underlying bronchial hyperresponsiveness to histamine and distilled water are reflected in their relationship to β_2-agonist use and daytime dyspnoea registered on diary cards and during bronchial challenge.

CONCLUSIONS

1. The standardized UNDW provocation test is a reliable, sensitive and reproducible method of assessing bronchial hyperresponsivleness in asthmatic subjects. The PD_{20}UNDW and sensitivity of the dose-response curve are the most sensitive thresholds reflecting the distilled water-induced bronchoconstrictor response.
2. The APS dosimeter technique in histamine challenge is a well reproducible method for the assessment of bronchial hyperresponsiveness. The dosimeter method is comparable to the 2-minute breathing technique with the Wright nebulizer, although the threshold values are not fully exchangeable.
3. Preinhalation of histamine induces refractoriness to UNDW-induced bronchoconstriction but not for the histamine-induced bronchoconstrictor response. The magnitude of refractoriness is related to the severity of bronchial hyperresponsiveness to histamine.
4. At a similar degree of bronchodilatation induced by high and low dose ipratropium bromide, high dose ipratropium bromide inhibits UNDW-induced bronchoconstriction. Hence, the response to inhaled UNDW is at least partly vagally mediated.
5. Eight weeks of treatment with beclomethasone, 800 μg daily, improves baseline lung function, morning and evening peakflow measurements, bronchial hyperresponsiveness to histamine and distilled water, and decreases daytime dyspnoea and $ß_2$-agonist use compared to baseline measurements and 8 weeks of treatment with nedocromil sodium, 16 mg daily, in atopic asthmatic subjects. Nedocromil sodium only improved bronchial hyperresponsiveness to histamine after 4 and 8 weeks of treatment.
6. Bronchial hyperresponsiveness to UNDW shows a closer and more consistent correlation to daytime dyspnoea and $ß_2$-agonist use registered on a diary card than histamine-induced bronchoconstriction. Furthermore, during distilled water challenge a significant correlation between increase in dyspnoea and fall in FEV_1 has been demonstrated, which is not the case during histamine challenge.

7. UNDW compared to histamine challenge is equally sensitive in detecting changes in bronchial hyperresponsiveness induced by anti-inflammatory drugs as beclomethasone.
8. During UNDW-induced bronchoconstriction increases in dyspnoea are correleted to decreases in FEV_1, but not during histamine challenge.

SAMENVATTING EN CONCLUSIES

In ***Hoofdstuk 1*** wordt de algemene inleiding gegeven van dit proefschrift met de hieraan ten grondslag liggende vraagstellingen.

Astma is een ziektebeeld dat gekarakteriseerd wordt door aanvallen van kortademigheid, piepen en hoesten als uiting van een toegenomen bronchiale hyperreaktiviteit. Deze bronchiale hyperreaktiviteit wordt gedefinieerd als een overmatige bronchusvernauwing van de luchtwegen, als reactie op blootstelling van die luchtwegen aan een kleine hoeveelheid aspecifieke prikkel, die in die hoeveelheid geen reactie veroorzaakt bij niet-astmatische proefpersonen. Bij patiënten met astma wordt een toename van ontstekingscellen in het longweefsel gezien. De mate van bronchiale hyperreaktiviteit is geassocieerd met de ernst van het ontstekingsproces in de luchtwegen, de variatie in longfunctie over de dag gemeten, met name het verschil in luchtweg-obstructie s'morgens en s'avonds, en de mate van reversibiliteit van de luchtweg-vernauwing na inhalatie met een $ß_2$-mimeticum, een luchtwegverwijdend medicament. De ernst van het astma, de klinische prognose van het astma met betrekking tot het ontwikkelen van chronisch obstructief longlijden en de mate van afname van de longfunctie zijn gerelateerd een de mate van bronchiale hyperreaktiviteit. Om deze bovengenoemde redenen staan het meten en de farmacologische beinvloeding van bronchiale hyperreaktiviteit centraal in dit proefschrift.

Bronchiale hyperreaktiviteit wordt veelal gemeten met bronchoprovocatie testen met histamine of methacholine. Histamine en methacholine zijn farmacologische prikkels die met name een direct constrictief effect hebben op de gladde spieren van de luchtwegen, in tegenstelling tot fysische prikkels die op meer indirecte wijze een bronchoconstrictie veroorzaken. Blootstelling aan fysische prikkels, veroorzaakt in hiervoor gevoelige patiënten het vrijkomen van mediatoren in de luchtwegwand, die op hun beurt een constrictie van de gladde spiercellen in de luchtwegen kunnen veroorzaken met een luchtwegvernauwing tot gevolg. Fysische prikkels meten dus een meer compleet traject van bronchiale hyperreaktiviteit dan farmacologische prikkels. Mede hierom wordt in de literatuur een betere correlatie tussen bronchoprovocatie tests met fysische prikkels en astmasymptomen gesuggereerd dan tussen farmacologische prikkels en astma-symptomen.

Verneveld gedistilleerd water is zo'n fysische prikkel, vergelijkbaar met inspanning. Gedistilleerd water is een specifieke prikkel voor het meten van bronchiale hyperreaktiviteit bij astmapatiënten daar bij normale proefpersonen, in tegenstelling tot de farmacologische prikkels, geen bronchoconstrictie kan worden bewerkstelligd.

De centrale vraagstelling in dit proefschrift is dan ook of fysische stimuli zoals gedistilleerd water voordelen hebben boven histamine in het meten van bronchiale hyperreaktiviteit bij astmapatiënten.

In ***Hoofdstuk 2*** onderzochten we bij negen astmapatiënten de reproduceerbaarheid van de histamine provocatietest uitgevoerd met het Astma provocatie systeem (APS), een inademings geactiveerde dosimeter. De histamine provocatietest met deze dosimeter werd uitgevoerd op twee verschillende dagen en liet een goede reproduceerbaarheid zien met een standaard deviatie voor herhaalde metingen van 10.4%.
De techniek met de APS dosimeter werd verder vergeleken met de methode volgens Hargreave, waarbij de patiënt via een Wright vernevelaar gedurende 2 minuten histamine inademt met rustademhaling. De twee methoden werden vergeleken bij een groep van vijftien patiënten met klachten van kortademigheid en verdenking op bronchiale hyperreaktiviteit. De methode met de Wright vernevelaar is een eenvoudige en goedkope methode, echter diverse factoren zoals de stroomsnelheid van de lucht door de jet-vernevelaar, de ademfrequentie en het adempatroon van de patiënt dienen gestandaardiseerd te worden voor het verkrijgen van reproduceerbare resultaten. De dosimeter techniek is een meer ingewikkelde en duurdere methode, met als voordeel dat de exacte dosis van de door de patiënt ingeademde histamine bekend is. Beide technieken toonden een goede correlatie ($r=0.93$, $p<0.0005$) en maakten in de zelfde mate onderscheid tussen responders en niet-responders. De limiet van overeenstemming, welke aangeeft uitgaande van een meting met de Wright vernevelaar in welke mate de APS methode hiervan kan afwijken (-102% and +83%) en het 95% betrouwbaarheid interval ($\pm$ 27%) geven echter aan dat de resultaten van de twee technieken niet geheel uitwisselbaar zijn.

In ***Hoofdstuk 3*** wordt een overzicht gegeven van de literatuur met betrekking tot de bronchoconstrictie geïnduceerd door inhalatie van ultrasoon verneveld gedistilleerd water.

Het onderliggende mechanisme van deze bronchoconstrictie wordt waarschijnlijk door diverse factoren bepaald. Het niet isotone karakter van het gedistilleerde water lijkt de uitlokkende factor te zijn welke aanleiding is tot bronchoconstrictie bij astmatische patiënten. Inhalatie van hypotone of hypertone aërosolen veroorzaakt geen reactie bij niet-astmatische proefpersonen. De zuurgraad en de mate waarin dat zuur titreerbaar is kunnen de gedistilleerd water geïnduceerde bronchoconstrictie versterken. Atropine en hoge doses ipratropium bromide, beiden anticholinergica, hebben een beschermend effect bij inhalatie van gedistilleerd water. Dit beschermend effect van atropine en ipratropium bromide suggereert dat het autonome zenuwstelsel tenminste gedeeltelijk betrokken is bij de bronchoconstrictie welke door inhalatie van gedistilleerd water wordt veroorzaakt. Mediatoren die vrij komen uit ontstekingscellen in de luchtwegen, zoals de mestcel, zijn ook bij deze reactie betrokken. Cromoglicinezuur en nedocromil, medicijnen die onder andere een stabilisatie van de mestcel kunnen geven en hiermee het vrijkomen van mediatoren voorkomen, kunnen een volledige bescherming geven voor gedistilleerd water geïnduceerde bronchoconstrictie. Ook de leukotrieën D_4 en E_4 spelen een rol in het onderliggend mechanisme, daar specifieke antagonisten de luchtweg vernauwende respons op inhalatie van gedistilleerd water kunnen doen afnemen.

De door inhalatie van verneveld gedistilleerd water veroorzaakte bronchoconstrictieve respons correleert met de respons veroorzaakt door inhalatie van histamine en methacholine bij volwassen patiënten. Bij kinderen is geen correlatie tussen beide responsen gevonden. Ook inspanning geïnduceerde bronchoconstrictie en bronchoconstrictie veroorzaakt door hyperventilatie met koude lucht is significant gecorreleerd aan de respons na inhalatie van gedistilleerd water, waarbij inspanning en gedistilleerd water de sterkste overeenkomst laten zien. Om deze reden wordt een gemeenschappelijk pathophysiologisch mechanisme bij inspanningsastma en de bronchoconstrictie door inhalatie van gedistilleerd water verondersteld.

Hoofdstuk 4 beschrijft het protocol van de bronchoprovocatie test met verneveld gedistilleerd water zoals het is toegepast in de in dit proefschrift beschreven studies. De reproduceerbaarheid van de dosis-respons curve werd onderzocht met een kortdurend interval van 2 à 3 weken bij 17 astmapatiënten en met een langdurig interval van ongeveer 3 maanden bij 16 astmapatiënten. Voor het vergelijken van de door inhalatie van gedistilleerd water geïnduceerde responsen werden diverse parameters, welke de semi-logaritmische dosis-respons curve beschrijven, zoals de drempelwaarde PD_{20}UNDW, het oppervlak onder de dosis-respons curve, de sensitiviteit en de reactiviteit, berekend. De PD_{20}UNDW, het oppervlak onder de dosis-response curve en de sensitiviteit, een lineare regressie van de dosis-response curve toonden een goede kortdurende en langdurende reproduceerbaarheid, waarbij de PD_{20}UNDW en sensitiviteit de kleinste standaard deviatie voor herhaalde metingen lieten zien. De reactiviteit, de hoek van het meest steile deel van de curve, was niet reproduceerbaar.

De tijdsduur van de bronchoconstrictie na het bereiken van de drempelwaarde PD_{20}UNDW werd onderzocht bij 12 astmapatiënten. Alhoewel er een grote variatie in spontaan herstel van de bronchoconstrictie bestond werd er een plateau van het bronchoconstrictieve effect bereikt tussen 30 seconden en 3 minuten na inhalatie, terwijl bij alle patiënten het spontane herstel binnen 5 minuten na inhalatie aanving.

Bij 16 atopische astmapatiënten werd de histamine respons vergeleken met die van verneveld gedistilleerd water. De PD_{20}UNDW en PD_{20}histamine toonde een significante correlatie ($r=0.72$, $p=0.002$).

In ***Hoofdstuk 5*** onderzochten we bij 9 astmapatiënten of inhalatie van histamine een refractaire periode kon veroorzaken voor een provocatietest met gedistilleerd water of een tweede histamine provocatietest. Tachyphylaxie of refractaire periode bij bronchoprovocatie tests is een fenomeen waarbij de mate van bronchoconstrictie na blootstelling aan een bepaalde stimulus afneemt, indien binnen enkele uren na de eerste test de patiënt opnieuw wordt bloot gesteld aan dezelfde stimulus. Dit fenomeen kan ook optreden bij verschillende stimuli. In ons onderzoek werd na een histamine provocatietest, nadat de FEV_1 spontaan hersteld was tot de basale uitgangswaarde, een tweede

provocatietest verricht met histamine of gedistilleerd water. Dit spontaan herstel trad binnen een uur op. Inhalatie van histamine veroorzaakte een tachyphylaxie voor inhalatie van gedistilleerd water. De gemiddelde PD_{20}UNDW nam toe van 3.5 $\pm$ 0.8 ml tot 11.8 $\pm$ 2.6 ml na inhalatie van histamine ($p<0.01$). Histamine inhalatie veroorzaakte geen tachyphylaxie voor een tweede provocatietest met histamine. De verandering in PD_{20}UNDW tengevolge van de tachyphylaxie was gecorreleerd aan de hoogte van de histamine drempel ($r=0.73$, $p<0.05$), wat aangeeft dat de mate van de door histamine veroorzaakt tachyphylaxie voor inhalatie van gedistilleerd water omgekeerd geassocieerd is met de ernst van bronchiale hyperreaktiviteit.

Hoofdstuk 6 beschrijft een studie naar de beschermende effecten van ipratropium bromide en terbutaline voor de bronchoconstrictie veroorzaakt door inhalatie van gedistilleerd water bij astmapatiënten. $ß_2$-mimetica, zoals salbutamol en fenoterol, medicijnen met mestcel stabiliserende eigenschappen zoals cromoglicinezuur en anticholinergica zoals atropine kunnen de bronchoconstrictie veroorzaakt door inhalatie van gedistilleerd water remmen of voorkomen. Ipratropium bromide, een anticholinergicum, heeft echter in de klinisch effectieve dosis van 40 of 80 μg geen effect op de door gedistilleerd water geïnduceerde luchtwegvernauwing. De beschermende effecten van ipratropium bromide zijn daarom niet duidelijk. In een gerandomiseerde, dubbel blinde, placebo gecontroleerde studie onderzochten we om deze reden, de beschermende effecten van ipratropium bromide 160 en 320 μg en terbutaline 500 μg voor gedistilleerd water geïnduceerde bronchusobstructie. Om de effecten van de medicatie te beoordelen, vergeleken we diverse parameters die de dosis-respons curve beschrijven, zoals de PD_{20}UNDW, de sensitiviteit en de reactiviteit. Beide medicijnen veroorzaakten een significante toename van de basale FEV_1 ($p<0.01$), waarbij er geen onderlinge verschillen waren tussen beide medicamenten en beide doses. Ipratropium 320 μg en terbutaline 500 μg gaven een remming van de door gedistilleerd water geïnduceerde bronchusobstructie, gemeten met de PD_{20}UNDW en sensitiviteit ($p<0.01$), terwijl de lage dosis ipratropium bromide geen protectie gaf. Er bestond geen correlatie tussen de mate van toename in FEV_1 en de mate van protectie, zodat het beschermende effect van de geteste medicijnen niet veroorzaakt wordt door hun luchtweg verwijdend

effect. Tevens kunnen wij concluderen dat het autonome zenuwstelsel tenminste ten dele betrokken is bij de luchtwegvernauwing tengevolge van inhalatie van gedistilleerd water.

In ***Hoofdstuk 7*** onderzochten we of de bronchiale hyperreaktiviteit van atopische astmapatiënten door medicatie beïnvloed kan worden. We vergeleken in een dubbel blinde, gerandomiseerde cross-over studie de effecten van nedocromil sodium, 4 maal daags 4 mg per inhalatie, met beclomethason dipropionaat, 4 maal daags 200 μg per inhalatie, bij 23 atopische astmapatiënten. Na een enkel-blinde placebo uitwas periode van drie weken, en na 4 en 8 weken behandeling met een dubbel-blind werkzaam medicament werden de effecten geëvalueerd met histamine en gedistilleerd water provocatietests, basale longfunctie, piekstroom metingen en β_2-agonist gebruik, bijgehouden met een dagboekje. Tijdens de behandelingsperiode met beclomethason werd een significante verbetering van de longfunctie ($p<0.005$) en de piekstroom meting gezien ($p<0.05$) en een afname van de bronchiale hyperreaktiviteit voor histamine ($p<0.0005$) en gedistilleerd water ($p<0.0005$). Nedocromil sodium verminderde de bronchiale overgevoeligheid voor histamine ($p<0.005$), maar niet voor gedistilleerd water, en er werden geen veranderingen in longfunctie en piekstroom metingen gevonden. Het β_2-agonist gebruik verminderde significant na 8 weken behandeling met beclomethason maar niet na behandeling met nedocromil sodium. De effecten van de behandeling met beclomethason, gemeten met histamine en gedistilleerd water provocatietests, basale longfunctie waarden, piekstroom metingen en β_2-mimeticum gebruik, waren superieur aan de effecten tijdens nedocromil sodium behandeling ($p<0.05$).

Na 8 weken behandeling met beclomethason verbeterde de histamine drempel nog significant ten opzichte van 4 weken behandeling, terwijl de PD_{20}UNDW niet verschillend was na 4 en 8 weken behandeling. De veranderingen in PD_{20}histamine en PD_{20}UNDW correleerden niet met elkaar na 4 weken behandeling, echter wel na 8 weken behandeling ($r_s = 0.64$, $p<0.05$). Dit verschil in tijdsduur waarin de veranderingen in bronchiale hyperreaktiviteit bereikt worden suggereert dat de stimuli histamine en gedistilleerd water bronchiale hyperreaktiviteit op een verschillend niveau meten. Deze veronderstelling wordt tevens ondersteund door wat bekend is over het onderliggende mechanisme.

Hoofdstuk 8 beschrijft de correlaties tussen astmaklachtenscores, β_2-mimeticum gebruik, en de bronchiale overgevoeligheid voor histamine en gedistilleerd water, tijdens de studie beschreven in hoofdstuk 7. De klachtenscores en het β_2-mimeticum gebruik werden door de patiënten in een dagboekje bijgehouden. Tevens werden astmasymptomen geregistreerd tijdens de histamine en gedistilleerd water bronchoprovocvatietests en gecorreleerd aan de veranderingen in longfunctie tijdens de tests.

De dyspnoeklachten overdag en het β_2-mimeticum gebruik lieten na 8 weken behandeling met beclomethason een significant sterkere afname zien dan tijdens behandeling met nedocromil sodium ($p=0.002$ voor beiden). De PD_{20}UNDW correleerde in geringe mate beter, maar met name meer consistent met de klachten van kortademigheid overdag en het β_2-mimeticum gebruik dan de histamine drempel met deze parameters correleerde.

De toename van dyspnoeklachten tijdens de bronchoprovocatietests liet een significante correlatie zien met de daling in FEV_1 tijdens inhalatie met gedistilleerd water maar niet tijdens inhalatie met histamine. Deze bevindingen geven aan dat verschillen in de pathophysiologische mechanismen van bronchiale hyperreaktiviteit voor histamine en gedistilleerd water, weerspiegelt worden in hun relatie met klachtenscores, zoals kortademigheidklachten overdag en β_2-mimeticum gebruik geregistreerd in een dagboekje en tijdens bronchoprovocatietests.

CONCLUSIES:

1. De gestandaardiseerde provocatietest met inhalatie van verneveld gedistilleerd water is een betrouwbare, sensitieve en reproduceerbare methode voor het meten van bronchiale hyperreaktiviteit in astmapatiënten. De PD_{20}UNDW en de sensitiviteit van de dosis-respons curve zijn de meest sensitieve drempelwaarden die de bronchoconstrictieve respons van gedistilleerd water weergeven.
2. De APS dosimeter techniek bij bronchoprovocatietests met histamine is een goed reproduceerbare methode voor het meten van bronchiale hyperreaktiviteit. De techniek met de dosimeter is goed vergelijkbaar met de inhalatietechniek met de Wright vernevelaar gedurende 2 minuten rustademhaling. De drempelwaarden tussen deze twee technieken zijn echter niet geheel uitwisselbaar.
3. Inhalatie van histamine voorafgaand aan een provocatietest met gedistilleerd water veroorzaakt een refractaire periode voor mist. Herhaalde provocatietests met inhalatie van histamine veroorzaakt geen tachyphylaxie voor histamine. De mate van de refractaire reactie van mist na histamine is gecorreleerd aan de histaminedrempel, en omgekeerd geassocieerd met de mate van bronchiale hyperreaktiviteit.
4. Inhalatie van hoge en lage dosis ipratropium bromide veroorzaken bij astmapatiënten een bronchodilatatie van dezelfde grootorde. Alleen de hoge dosis ipratropium bromide is in staat de bronchoconstrictie veroorzaakt door inhalatie van gedistilleerd water te verminderen. Hieruit volgt dat het autonome zenuwstelsel tenminste ten dele een rol speelt in de bronchoconstrictieve respons van de luchtwegen op inhalatie van gedistilleerd water bij astmapatiënten.
5. Inhalatie van beclomethason, 800 ug per dag, gedurende acht weken verbetert de longfunctie, de piekstroom metingen s'morgens en s'avonds, de bronchiale hyperreaktiviteit voor histamine en verneveld gedistilleerd water en vermindert de dyspnoeklachten overdag en het $ß_2$-mimeticum gebruik, in vergelijking met drie weken placebo medicatie en nedocromil sodium, 16 mg per dag, gedurende acht weken.

6. Bronchoprovocatie met gedistilleerd water, vergeleken met die met histamine, is gelijkwaardig sensitief in het meten van veranderingen in bronchiale hyperreactiviteit tengevolge van anti-inflammatoire medicijnen, zoals beclomethason.

7. Bronchiale hyperreaktiviteit voor verneveld gedistilleerd water heeft een betere en meer consistente associatie met dyspnoeklachten overdag en β_2-mimeticum behoefte, geregistreerd in een dagboekje dan de histaminedrempel met deze parameters heeft.

8. Tijdens bronchoprovocatie met verneveld gedistilleerd water bestaat er een correlatie tussen de toename van dyspnoeklachten en de afname van FEV_1, welke tijdens bronchoprovocatie met histamine niet aanwezig is.

LIST OF ABBREVIATIONS

AUC	:	Area under the dose-response curve
FEV_1	:	Forced expiratory volume in 1 second
MEF_{50}	:	Maximal expiratory flow at 50% of the vital capacity
MEF_{25}	:	Maximal expiratory flow at 25% of the vital capacity
PC_{20}histamine	:	Provocative concentration of histamine causing a 20% fall in FEV_1
PD_{20}histamine	:	Provocative dose of histamine causing a 20% fall in FEV1
PD_{20}UNDW	:	Provocative cumulative dose of UNDW causing a 20% fall in FEV1
PO_{20}UNDW	:	Provocative nebulizer output causing a 20% fall in FEV1 during UNDW challenge
UNDW	:	Ultrasonically nebulized distilled water
SD	:	Standard deviation
SE	:	Standard error

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De auteur van dit proefschrift werd geboren op 29 september 1955 te Weststellingwerf. Hij volgde zijn middelbare school opleiding Atheneum B aan het Canisius College te Nijmegen van 1968 tot 1974. In augustus 1974 begon hij zijn studie Geneeskunde aan de Katholieke Universiteit te Nijmegen, waarbij hij in september 1982 zijn Artsexamen behaalde.

Hij vervulde zijn militaire dienst plicht van november 1982 tot mei 1984 waarbij hij gedetacheerd was op de afdeling Hematologie van het St. Radboud ziekenhuis te Nijmegen (hoofd: Prof. Dr. C. Haanen). Gedurende deze periode was hij werkzaam op de plasmaferese afdeling en verrichtte hij wetenschappelijk onderzoek op het gebied van flowkaryotypie.

Op 1 juni 1984 startte zijn opleiding Interne Geneeskunde in het St. Radboud ziekenhuis (opleider: Prof. Dr. A. van 't Laar). Van 1 maart 1987 tot 1 maart 1990 volgde hij de opleiding Longziekten in het Universitair Longcentrum Nijmegen (opleider: Prof. Dr. C.L.A. van Herwaarden). Op 1 maart 1990 werd hij geregistreerd als longarts. Het wetenschappelijk onderzoek dat tot deze dissertatie leidde werd verricht tijdens zijn opleiding en de twee daarop volgende jaren in het Academisch ziekenhuis Nijmegen.

Sinds 1 april 1992 is hij werkzaam als longarts in het ziekenhuis Leyenburg te Den Haag. Hij is gehuwd met Jacqueline Loonen en vader van twee zonen: Pieter en Maarten.

Behorende bij het proefschrift

Bronchial Hyperresponsiveness to Ultrasonically Nebulized Distilled Water and Histamine in Asthmatic subjects

C.A.R. Groot

Nijmegen, 29 september 1992

1. Inhalatie van histamine voorafgaand aan de inhalatie van ultrasoon verneveld gedistilleerd water kan bij astmapatiënten een refractaire periode veroorzaken voor de door gedistilleerd water geïnduceerde bronchoconstrictie, waarbij de mate van deze tachyphylaxie omgekeerd evenredig is met de mate van bronchiale hyperreactiviteit (dit proefschrift).

2. Het beschermende effect van ipratropium bromide voor bronchusobstructie, geïnduceerd door de inhalatie van ultrasoon verneveld gedistilleerd water, is dosis afhankelijk en is niet gecorreleerd aan de mate van luchtwegverwijding veroorzaakt door dit medicament (dit proefschrift).

3. De behandeling van matig ernstige astmapatiënten met beclomethason in een dagdosis van 800 μg gedurende 8 weken, is superieur aan de behandeling met nedocromil 16 mg daags (dit proefschrift).

4. Astmasymptoomscores correleren beter met bronchiale hyperreactiviteit voor ultrasoon verneveld gedistilleerd water dan met bronchiale hypperreactiviteit voor histamine (dit proefschrift).

5. Astmapatiënten hebben tijdens provocatietests met ultrasoon verneveld gedistilleerd water een betere perceptie van bronchusobstructie dan tijdens provocatie met histamine (dit proefschrift).

6. Het ovarieel hyperstimulatie syndroom lijkt bij daartoe gevoelige patiënten astma te kunnen uitlokken. Bij het ziektebeeld worden gelijksoortige mediatoren vrijgemaakt. C. Groot et al. Lancet 1991;337:112

7. Bij patiënten die deelnemen aan longrevalidatie spelen psychosociale problemen vaak een centrale rol bij het instand houden van hun ziek zijn (eigen waarneming).

8. De arts die een kind met een "eenvoudige" Wilms'tumor niet naar een kinderoncologisch centrum verwijst, dient zich te realiseren dat de kans op genezing van een recidief uitermate klein is. Ook voor deze patiënt geldt: "There is no second chance". J.J. Groot-Loonen et al. Arch Dis Childh 1990;65:968-970.

9. De arts onderschat vaak zijn voorbeeldfunctie met betrekking tot eet-, drink- en rookgewoonten.

10. Een onderzoeker die begint aan een promotie-onderzoek is nog lang niet jarig.

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